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(54) Title: COMPOSITIONS AND METHODS FOR CONTROLLED-RELEASE DELIVERY AND INCREASED POTENCY OF PHARMACEUTICALS VIA HYDROPHOBIC ION-PAIRING

(57) Abstract: The present invention relates to compositions and methods for improved efficacy of pharmaceutical compounds and controlled-release drug delivery systems. Using the process of hydrophobic ion pairing, antimicrobials having improved antimicrobial activity and anticancer agents having improved cytotoxic activity, as well as other pharmaceuticals, are provided. The present invention also relates to the use of poloxamer gels to deliver hydrophobic ion-paired pharmaceuticals.

COMPOSITIONS AND METHODS FOR CONTROLLED-RELEASE DELIVERY AND INCREASED POTENCY OF PHARMACEUTICALS VIA HYDROPHOBIC ION-PAIRING

This application claims priority benefit to U.S. Provisional Patent Application No. 60/162,822, filed November 1, 1999, U.S. Provisional Patent Application No. 60/231,257, filed September 9, 2000, and U.S. Provisional Patent Application No. 60/231,374, filed September 9, 2000.

This invention was made during the course of work supported by the United States Government, under the National Institutes of Health. As such, the United States Government may have certain rights to this invention.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for improved efficacy of pharmaceutical compounds and controlled-release drug delivery systems. Antimicrobials having improved antimicrobial activity and anticancer agents having improved cytotoxic activity are provided using the process of hydrophobic ion pairing. The present invention also relates to the use poloxamer gels to deliver hydrophobic ion-paired pharmaceuticals.

BACKGROUND OF THE INVENTION

Many drug therapies require controlled and sustained release of the pharmaceutical product to a patient to optimize effectiveness of the pharmaceutical. These pharmaceutical products, which include bioactive proteins or peptides, as well as small molecule drugs, are typically administered systemically. Administration of these drugs may be parenteral (*e.g.*, intravenous, intramuscular or subcutaneous) or enteral (*i.e.*, by mouth). Many types of drug delivery are faced with a significant limitation in that there is an initially high level of drug provided to the patient immediately following administration, but there is no constant and controlled release of the drug (*i.e.*, where the level of the drug remains constant over an extended period of time). However, some diseases are best managed by the maintenance of constant and sustained levels of a therapeutic compound, or through delivery of a drug to a localized area, such as an organ or the site of a wound. Thus, traditional parenteral administration does not provide optimum treatment. For example, insulin is typically administered parenterally via subcutaneous injection. Ideally, the level of exogenous insulin

administered to a diabetic patient will mimic the physiological levels of insulin observed in non-diabetics. Unfortunately, the presently used techniques of subcutaneous administration do not provide the desired extended and sustained release of insulin to the patient.

The demand for more sophisticated pharmaceutical delivery systems is increasing in view of advances in understanding of the molecular basis of disease. As the genes and proteins involved in diseases are identified, there is an increasing demand to develop methods for effective administration of those proteins (as well as small molecules which specifically regulate those proteins) for the treatment of disease. Many pharmaceuticals suffer from poor stability, rapid turnover from the body (*i.e.*, short half lives) following administration, and poor solubility in physiological conditions, all of which diminish drug efficacy. Rapid turnover of a pharmaceutical also results in the necessity for more frequent administrations and/or larger doses, resulting in increased toxicity, side effects, inconvenience, discomfort and/or expense to the patient.

Furthermore, for those drugs which display toxicity to the patient, methods for making a drug more efficacious will permit lower administration doses and/or less frequent administration, thereby reducing toxicity and patient discomfort. Thus, what is needed are compositions and methods for improving the longevity, potency and/or stability of pharmaceuticals in physiological conditions. There is a need for compositions and methods that permit less frequent and more consistent dosing of pharmaceuticals.

One such disease that would benefit from improved efficacy and delivery methods are infections. Infections represent a significant health risk, which is acute in some groups of patients, including hospitalized individuals, as well as those with underlying disease conditions and/or immune defects. In particular, infections represent a serious risk to diabetic patients. These patients often experience slow and/or incomplete wound healing, ulceration of the extremities, and are prone to infection. These ulcers in diabetic patients are often open wounds, and can encompass both soft tissue and the underlying bone. The problem is widespread, as approximately 15% of all diabetics will develop these ulcers. Infections within these ulcers are difficult to effectively treat, due to poor circulation at these sites (*i.e.*, limiting the potential for systemically administered antimicrobial treatments to reach the wound sites). In extreme cases, limb amputation becomes necessary (accounting for half of all amputations done in this country), and infected wounds can be life threatening.

Current treatment of diabetic wounds or ulcers consists of debridement, packing the wound with gauze, and placing the patient on systemic antimicrobials. However, no evidence exists that an adequate amount of drug reaches the wound site with this treatment. Indeed, there remains a need for compositions and methods for improved treatment and prevention of infection. In particular, there is a need for compositions and methods suitable for use in treating or preventing infections in diabetic patients.

The treatment of cancer would also benefit from improved methods of delivery and improved drug efficacy. Chemotherapy, either for treating cancer or other diseases (*e.g.*, diseases of the peritoneal cavity) is often accompanied by significant toxicity. For example, cis-diamminedichloroplatinum (II) (cisplatinum diammine dichloride; cisplatin) is a widely used chemotherapeutic agent for the treatment of many different cancers. However, due to the risk of neurotoxicity and nephrotoxicity, its clinical application is limited to low-dose levels.

Thus, what is needed are compositions comprising cisplatin that are capable of being more bioavailable. Indeed, what methods and compositions are needed in the art are those which provide efficient, safe and effective treatment of disease.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for improved efficacy of pharmaceutical compounds and controlled-release drug delivery systems by forming a hydrophobic ion-paired (HIP) surfactant complex. In preferred embodiments, antimicrobials having improved antimicrobial activity and anticancer agents having improved cytotoxic activity are synthesized using the process of hydrophobic ion pairing (HIP). In other embodiments, the present invention provides methods for metal-assisted hydrophobic ion pairing, the use of poloxamer gels to deliver hydrophobic ion-paired pharmaceuticals, and methods to treat a subject using the compositions provided by the present invention.

The present invention provides methods for preparing pharmaceuticals with controlled-release properties, where the pharmaceutical is in a hydrophobic ion-paired complex with an amphiphilic counterion, where the complex has reduced aqueous-solubility, and further where the hydrophobic ion-paired complex is isolated.

In one preferred embodiment, the hydrophobic ion-paired complex is dried to form a pharmaceutical powder. In another embodiment, the invention provides the controlled-release pharmaceutical produced by the method.

In one embodiment, the present invention provided a method for preparing pharmaceuticals with controlled-release properties, where the pharmaceutical is an antimicrobial. In another preferred embodiment, the pharmaceutical used in the method is selected from vancomycin, tetracycline, tacrine, L-phenylephrine, cisplatin, isoniazid and isoniazid methanesulfonate.

In another embodiment, the present invention provides a method for preparing pharmaceuticals with controlled-release properties, where the pharmaceutical is in a hydrophobic ion-paired complex with an amphiphilic counterion, and where the amphiphilic counterion is selected from bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate (SDS), sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide (CTAB), palmitoylcholine chloride, palmitoyl carnitine chloride (PCC), ZWITTERGENT 3-16, polyethylene oxide polymer (POLOX 600K), tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide,trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane, 1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

In still another embodiment, the invention provides a composition for pharmaceutical delivery comprising a hydrophobic ion-paired complex and a poloxamer solution having reverse thermal gelation properties. In a preferred embodiment, the poloxamer is poloxamer 407.

The present invention provides a method for preparing a hydrophobic ion-paired complex comprising a neutral pharmaceutical or a zwitterionic pharmaceutical, and further where the hydrophobic ion-paired complex is formed by metal-assisted hydrophobic ion-pairing.

In one preferred embodiment, the metal-assisted hydrophobic ion-paired complex is dried to form a pharmaceutical powder.

In another embodiment, the present invention provides a method for preparing pharmaceuticals with controlled-release properties, where the pharmaceutical is in a metal-assisted hydrophobic ion-paired complex with an amphiphilic counterion, and where the amphiphilic counterion is selected from bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate (SDS), sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide (CTAB), palmitoylcholine chloride, palmitoyl carnitine chloride (PCC), ZWITTERGENT 3-16, polyethylene oxide polymer (POLOX 600K), tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide,trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane, 1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

In one embodiment, the pharmaceutical in the metal-assisted hydrophobic ion-paired complex is isoniazid. In another embodiment, the pharmaceutical in the metal-assisted hydrophobic ion-paired complex is a polypeptide.

In other embodiments, the metal ion in the metal-assisted hydrophobic ion-paired complex is multivalent. In another embodiment, the metal ion is divalent. In another embodiment, the metal ion is calcium or zinc.

In one embodiment, the present invention provides a metal-assisted hydrophobic ion paired complex.

In still another embodiment, the invention provides a composition for pharmaceutical delivery comprising a metal-assisted hydrophobic ion-paired complex and a poloxamer solution having reverse thermal gelation properties. In a preferred embodiment, the poloxamer is poloxamer 407.

The present invention provides a hydrophobic ion-paired complex comprising an antimicrobial. In one preferred embodiment, the hydrophobic ion-paired complex is dried to form a pharmaceutical powder. In another embodiment, the invention provides a hydrophobic ion-paired complex comprising an antimicrobial where the antimicrobial is selected from the group tetracycline, vancomycin, isoniazid and isoniazid methanesulfonate.

In still another embodiment, the invention provides a composition for pharmaceutical delivery comprising a hydrophobic ion-paired complex comprising an antimicrobial and a poloxamer solution having reverse thermal gelation properties. In a preferred embodiment, the poloxamer is poloxamer 407.

The present invention provides a method for preparing a hydrophobic ion-paired complex comprising a pharmaceutical with improved efficacy. In one preferred embodiment, the metal-assisted hydrophobic ion-paired complex is dried to form a pharmaceutical powder. In another embodiment, the present invention provides a pharmaceutical with improved efficacy.

In one embodiment, the pharmaceutical in the hydrophobic ion-paired complex is an anti-cancer agent or an antimicrobial. In one embodiment, the pharmaceutical is selected from vancomycin, tetracycline, tacrine, L-phenylephrine, cisplatin, isoniazid or isoniazid methanesulfonate.

In another embodiment, the present invention provides a method for preparing pharmaceuticals with improved efficacy, where the pharmaceutical is in a metal-assisted hydrophobic ion-paired complex with an amphiphilic counterion, and where the amphiphilic counterion is selected from bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate (SDS), sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide (CTAB), palmitoylcholine chloride, palmitoyl carnitine chloride (PCC), ZWITTERGENT 3-16, polyethylene oxide polymer (POLOX 600K), tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide,trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

In still another embodiment, the invention provides a composition for improved pharmaceutical efficacy, comprising a hydrophobic ion-paired complex and a poloxamer solution having reverse thermal gelation properties. In a preferred embodiment, the poloxamer is poloxamer 407.

The present invention provides a method for treating a subject, which comprises a hydrophobic ion-paired complex and a poloxamer solution. In one embodiment, the hydrophobic ion-paired complex comprises an antimicrobial. In another embodiment, the hydrophobic ion-paired complex comprises an antimicrobial selected from tetracycline, vancomycin, isoniazid and isoniazid methanesulfonate.

In one embodiment, the localized region of the subject for treatment is selected from a diabetic ulcer, a surgical incision site, a wound. In another embodiment, the localized region is selected from the group consisting of sites external to the body of said subject and sites internal to the body of said subject.

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

As used herein, the term "antimicrobial" is used in reference to any compound which inhibits the growth of, or kills microorganisms. It is intended that the term be used in its broadest sense, and includes, but is not limited to compounds such as antibiotics which are produced naturally or synthetically. It is also intended that the term includes compounds and elements that are useful for inhibiting the growth of, or killing microorganisms.

As used herein, the terms "drug" and "pharmaceutical" refers to any molecule of any composition, including protein, peptide, nucleic acid, organic molecule, inorganic molecule, or combinations of molecules, biological or non-biological, which are capable of producing a physiological response and are suitable for administration to a subject to treat or prevent pathology (*e.g.*, disease). As used herein, a drug or pharmaceutical provide at least one beneficial response in the cure, mitigation, treatment or prevention of a disease, condition or disorder (*e.g.*, to treat a bacterial infection). Herein, the terms "drug" and

"pharmaceutical" are used interchangeably. It is intended that theses terms include, but not be limited to, such compounds as antimicrobials, anti-cancer compounds, and other compounds suitable for use in treating and/or preventing any type of pathology.

As used herein, the expressions "controlled-release," "sustained-release," "delayed-release," "prolonged-release," "extended-release," and "depot-delivery" are all used synonymously. These expressions refer to the delivery of a pharmaceutical in which the delivery composition or method provides some aspect of prolonged or extended release of the pharmaceutical to the biological system as compared to the delivery of that pharmaceutical alone without incorporation into a "controlled-release" composition or method. For example, hydrophobic ion-pairing and poloxamer drug delivery methods both impart controlled-release features to a pharmaceutical.

As used herein, a "zwitterion" is any molecule containing both positive and charges at physiological conditions (*i.e.*, physiological ionic concentrations and pH).

As used herein, the term "amphiphilic" refers to any molecule having a water soluble polar head (hydrophilic) and a water insoluble organic tail (hydrophobic).

As used herein, the terms "systemically active drug" and "systemically active agent" are used broadly to indicate a substance or composition which will produce a pharmacologic response at a site remote from the point of application.

As used herein, the term "topically" means application of a composition to the surface of the skin, mucosa, viscera, etc.

As used herein, the terms "topically active drug" and "topically active agent" indicates a substance or composition which elicits a pharmacologic response at the site of application.

As used herein, "PBS" refers to phosphate buffered saline solution, which contains 0.1 M NaCl and 20 mM phosphate buffer at pH 7.4.

As used herein, the term "poloxamer" refers to a copolymer of poly(oxyethylene) and poly(oxypropylene) (*See*, U.S. Pat. Nos. 4,100,271 and 5,457,093, both of which are herein incorporated by reference).

As used herein, the term "poloxamer gel" refers to a gel comprised of a copolymer of poly(oxyethylene) and poly(oxypropylene). In some embodiments, the poloxamer composition of the present invention transitions from a gel to a fluid at and vice versa at temperatures about between 18-20°C. It is recognized that the transition temperature vary

by the presence of other solutes in the poloxamer solution (*e.g.*, salts [*i.e.*, ions], carbohydrates or proteins).

As used herein, the term "PLURONIC" refers to poloxamers sold under the trade name PLURONIC.

As used herein, the term "fluid" refers to any composition capable of flowing.

As used herein, the term "therapeutic amount" refers an amount of a drug (*e.g.*, an antimicrobial drug) that produces a beneficial effect (*e.g.*, eradicates or inhibits a bacterial infection).

As used herein, the term "subject" refers to a human or other animal that is capable of being treated with a pharmaceutical composition.

As used herein, the term "cancer" refers to a malignant neoplasia characterized by the uncontrolled growth of anaplastic cells that tend to invade surrounding tissue and may develop the ability to metastasize to distant body sites.

As used herein, the term "suspected of having cancer" refers to a patient or subject who has been diagnosed as having cancer or tested positive in one or more tests diagnostic for cancer.

As used herein, the term "susceptible to cancer" refers to a subject or patient having one or more risk factors for cancer, is currently being treated for cancer, or is in remission from a previously diagnosed cancer.

As used herein, the term "post-operative for removal of a cancer" means the period of time after a tumor has been removed from a subject.

As used herein, the term "excision cavity" refers to an area of the body from which a tumor has been removed.

As used herein, a "wound" may constitute a variety of insults or damage to body tissues, for example a wound may involve a laceration, cut or scrape, surgical incision, sore, thermal burn, puncture, or decubitus ulcer (*e.g.*, bedsores). Wounds can be classified in one of two general categories, namely either partial thickness or full thickness wounds. A partial thickness wound is limited to the epidermis and superficial dermis with no damage to the dermal blood vessels. A full thickness wound involves loss of the dermis and extends to deeper tissue layers and involves disruption of the dermal blood vessels.

As used herein, the term "cytotoxic drug" typically, but not exclusively, refers to a chemotherapeutic agent useful in treating cancer that is cytotoxic (*i.e.*, detrimentally effects or kills cells), particularly to rapidly dividing cells.

As used herein, the term "LD_{50}" refers to the concentration at which 50% cytotoxicity measured as lethality to cells is observed. Cytotoxicity can be measured by the method of Alley *et al.*, Cancer Res. 48: 589-601 (1988) or Scudiero *et al.*, Cancer Res., 48:4827 (1988). However, it is not intended that the present invention be limited to any particular method of measurement. In one embodiment, the LD_{50} is based on the drug concentration at which a 50% reduction in the activity of mitochondrial enzymes is observed.

As used herein, the term "sample" is used in its broadest sense. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include, but are not limited to, blood products, such as plasma, serum and the like, and tissue samples, such as biopsy material. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. These examples are not to be construed as limiting the sample types applicable to the present invention.

DESCRIPTION OF THE FIGURES

Figure 1 provides a graph showing the dissolution and release of tacrine from various hydrophobic ion-paired complexes *in vitro* in PBS as a function of time.

Figure 2 provides a graph showing the log of the partitioning coefficients (*P*, where *P*=[organic]/[aqueous]) of various hydrophobic ion-paired complexes of L-phenylephrine as a function of the hydrophilic-lypophilic balance number (HBN) of the surfactant used to make the hydrophobic ion-paired complex.

Figure 3 provides the chemical structures of isoniazid and isoniazid methanesulfonate, and the reaction scheme for metal-assisted hydrophobic ion-pairing using isoniazid.

Figure 4 provides a graph showing the log of the partitioning coefficients (*P*, where *P*=[organic]/[aqueous]) of isoniazid, isoniazid methanesulfonate (IHMS), and various hydrophobic ion-paired complexes of IHMS produced using cationic surfactants.

Figure 5 is a growth curve for *E. coli* exposed to tetracycline and tetracycline-AOT complex (as well as control cultures).

Figure 6 is a growth curve for *E. coli* exposed to tetracycline and tetracycline- AOT complex (as well as control cultures).

Figure 7 is a growth curve for *E. coli* exposed to tetracycline and tetracycline-AOT complex (as well as control cultures).

Figure 8 is a growth curve for *E. coli* exposed to tetracycline and tetracycline-AOT complex (as well as control cultures).

Figure 9 provides a histogram showing the intracellular platinum concentrations in Chinese hamster ovary (CHO) cells exposed to either cisplatin or a cisplatin-AOT complex.

Figure 10 provides a graph showing the cell survival rates of Chinese hamster ovary (CHO) cells exposed to either cisplatin or a cisplatin-AOT complex.

Figure 11 provides a graph showing the vancomycin levels in mouse plasma following subcutaneous delivery of a poloxamer gel containing vancomycin or a vancomycin-AOT complex, and a control injection of water containing vancomycin-AOT.

Figure 12 provides a graph showing the cumulative amount (%) of vancomycin released over time into PBS from a vancomycin-AOT complex.

Figure 13 provides a graph showing the cumulative amount (%) of vancomycin released over time into PBS from a vancomycin-AOT complex.

DESCRIPTION OF THE INVENTION

The present invention relates to the process of "hydrophobic ion pairing" (HIP), in which a drug (*i.e.*, a pharmaceutical product or other biological agent) is non-covalently and reversibly modified to yield a drug species with advantageous properties. It is not intended that the present invention be limited to any particular drug. Indeed, it is contemplated that numerous drugs will find use with the HIP process of the present invention. It is contemplated that the HIP process and applications of this process will find widespread use in the pharmaceutical industry, and in particular, will find use in improving or extending the usefulness of previously characterized pharmaceuticals. The HIP process is useful with any number of compounds, including small-molecule drugs as well as peptides and proteins. The present invention provides HIP-complexes containing a desired pharmaceutical that demonstrates improved drug delivery, controlled-release properties, and improvements in drug efficacy. Also, the present invention provides improved methods for HIP-complex formation.

HIP complex formation involves the stoichiometric replacement of the small counterion(s) complexed with a drug in aqueous solution with an ionic detergent (*i.e.*, an amphiphilic molecule or surfactant) of similar charge. Both anionic and cationic surfactants

find use with the present invention to form HIP complexes with cationic and anionic drugs, respectively. HIP complexes can be formed by a variety of methods, including precipitation ion pairing, two-phase emulsion, and monophase processing, all of which are known in the art. In some embodiments in which both the surfactant and the drug are water soluble, precipitation ion pairing is the most convenient of these three methods.

HIP-drug complexes are formed by the stoichiometric replacement of the small, charged counter ions, such as chloride ions, which typically associate with larger charged molecules in an aqueous environment, with ionic surfactants at an appropriate molar ratio (Kendrick *et al.*, *Arch. Biochem. Biophys.*, 347:113-118 [1997]; Matsuura *et al.*, *JACS* 115:1261-1264 [1993]; Meyer *et al.*, *Biopolymers* 35:451-456 [1995]; Meyer *et al.*, *Int. J. Peptide Res.*, 47:177-181 [1996]; Meyer and Manning, *Pharm. Res.*, 15(2):188-193 [1998]; Powers *et al.*, *Biopolymers* 33:927-32 [1993]; and U.S. Patent Nos. 5,770,559 and 5,981,474, herein incorporated by reference). The formation of a drug-surfactant complex using the HIP process is distinct from the mechanisms of liposome or micelle formation, and results in a complex that has greatly reduced aqueous-solubility (this reduction often reaches a factor of 50-100 fold), and causes the precipitation of the complex from the aqueous solution in which it is synthesized. This precipitate can be isolated, washed and dried to form pharmaceutical powders which find use as delivery vehicles (Matsuura *et al.*, *JACS*, 115:1261-1264 [1993]; Powers *et al.*, *Biopolymers* 22:927-932 [1993]; and Meyer *et al.*, *Biopolymers* 35:451-456 [1995]; Meyer and Manning, *Pharm. Res.*, 15(2):188-193 [1998]; and U.S. Patent Nos. 5,770,559 and 5,981,474, herein incorporated by reference).

While HIP-drug complexes display reduced aqueous solubility, these same complexes demonstrate increased solubility in low-dielectric organic solvents, sometimes by orders of magnitude. This technique makes it possible to obtain true homogeneous solutions of ionic compounds in neat organic solvents with the only requirement being an accessible charge on the drug molecule. Surprisingly, it has been shown that HIP-complexed proteins retain their structural integrity (*i.e.*, secondary and tertiary structure) and enzymatic activity while dissolved in organic solvents, and also retain activity following the dissociation from the surfactant and return to the aqueous phase (Bromberg and Klibanov, *Proc. Natl. Acad. Sci. USA* 91:143-147 [1994]; Matsuura *et al.*, *JACS* 115:1261-1264 [1993]; Meyer *et al.*, *Biopolymers* 35:451-456 [1995]; Meyer *et al.*, *Int. J. Peptide Res.*, 47:177-181 [1996]; Meyer and Manning, *Pharm. Res.*, 15(2):188-193 [1998]; and Powers *et al.*, *Biopolymers* 33:927-32 [1993]).

HIP-complex solubility in organic solvents is an advantageous feature in the preparation of some pharmaceutical compositions provided by some embodiments of the present invention. For example, HIP used in conjunction with precipitation by a compressed antisolvent technique (PCA), has been shown to be effective in circumventing problems associated with microscale phase separation and consequent burst effects, and low incorporation yields in polymer [L-lactide] (PLA) microparticle processing (*See e.g.*, Falk *et al.*, *J. Controlled Release* 44:77-85 [1997]; Manning *et al.*, *Biotechnol. Bioengin.*, 48:506-512 [1995]; U.S. Patent No. 5,770,559, and PCT WO 99/47543, herein incorporated by reference). Thus, the HIP compositions and methods provided by the present invention find use with the synthesis of pharmaceutical compositions.

Despite the use of ion-paired complexes in the preparation of pharmaceutical compositions, it is a widely held belief in the art that there is no benefit to ion-paired complexes provide no benefits that would improve the bioavailability characteristics of a pharmaceutical composition, as it is commonly believed that these complexes dissociate very quickly under physiological conditions. Thus, the present invention provides surprising properties and advantages.

Numerous ionic surfactants find use with the HIP process and the present invention. The anionic detergents sodium dodecyl sulfate (SDS) and sodium bis(2-ethylhexyl) sulfosuccinate (AOT; sodium dioctyl sulfosuccinate) are the most commonly used surfactants in HIP methods, but other surfactants have also been successfully utilized, including arginine octyl ester, arginine dodecyl ester, cholesterol sulfate, sodium laurate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide (CTAB), palmitoylcholine chloride, palmitoyl carnitine chloride (PCC), ZWITTERGENT 3-16, and polyethylene oxide polymer (POLOX 600K) (Bromberg and Klibanov, *Proc. Natl. Acad. Sci. USA* 91:143-147 [1994]; Choi *et al.*, *Int J Pharm.*, 203(1-2):193-202 [2000]; Khossravi, *Int. J. Pharm.*, 155:179-190 [1997]; and PCT WO 99/47543, herein incorporated by reference). Thus, it is not intended that the present invention be limited to any particular surfactants. Indeed, it is contemplated that a wide variety of surfactants will find use with the present invention.

In some embodiments, the present invention provides HIP-complexed pharmaceuticals. In particularly preferred embodiments, these pharmaceuticals include antimicrobials (*e.g.*, vancomycin, tetracycline and isoniazid), chemotherapy agents (*e.g.*, cisplatin), drugs used in the treatment of Alzheimer's disease (*e.g.*, tacrine), and

decongestants (*e.g.*, L-phenylephrine). As indicated herein, HIP-complexed drugs have improved efficacy and controlled (*i.e.*, sustained or prolonged) release properties when used alone, as well as when they are incorporated into another delivery vehicle, such as poloxamer gel. The present invention also provides improved methods for hydrophobic ion pairing for the complexation of neutral or zwitterionic compounds, as well as HIP-complexes incorporating a covalently modified drug. However, it is not intended that the present invention be limited to the use of the drugs listed herein, as the invention finds use with numerous pharmaceuticals.

The remainder of the Description of the Invention is divided into the following sections:

I) Synthesis of a Hydrophobic Ion Paired Drug Complexes
II) Metal-Assisted Hydrophobic Ion Pairing
III) Improved Drug Efficacy by Hydrophobic Ion Pairing
IV) Use of Covalently-Modified Drugs in Hydrophobic Ion Pairing
V) Use of Hydrophobic Ion Paired Drugs for Controlled Drug Release
VI) Delivery of Hydrophobic Ion Paired Drugs Using Poloxamer Gels

I) Synthesis of Hydrophobic Ion-Paired Drug Complexes

As indicated previously, the present invention provides compositions and methods for the non-covalent modification of pharmaceuticals to produce HIP complexes. The formation of HIP complexes involves the stoichiometric replacement of the small counter ion(s) associated with the pharmaceutical in physiological (or other ionic) conditions with an ionic surfactant of like charge. The formation of an HIP complex results in a reduction in aqueous solubility of the substrate (this reduction often reaches a factor of 50-100 fold), while at the same time significantly increasing its solubility in organic solvents. The pharmaceutical HIP complex precipitates when formed in aqueous solutions. In some embodiments, the resulting precipitate is collected, washed and dried to form a pharmaceutical powder.

It is not intended that the present invention be limited to any one protocol for HIP complex formation, nor any particular drug or surfactant used in the HIP process, as numerous other pharmaceuticals and reagents find use with the present invention. The HIP complexes, as well as compositions comprising the complexes of the present invention are useful in various treatment regimens.

Some embodiments of present invention provide HIP-complexed antimicrobials, including tetracycline-AOT, vancomycin-AOT, isoniazid-AOT, and a covalently modified form of isoniazid, isoniazid methanesulfonate (IHMS), which is incorporated into HIP complexes using a number of cationic surfactants. However, it is not intended that the present invention be limited to these particular antimicrobials, as various other antimicrobials find use in the present invention.

In some embodiments, an HIP-complexed form of tetracycline, tetracycline-AOT, is provided as described in Example 1. Briefly, in some embodiments, equimolar aqueous stock solutions were prepared for tetracycline hydrochloride (Sigma) and the anionic surfactant dioctylsulfosuccinate sodium salt, Aerosol OT (AOT; Sigma). Equal volumes of these solutions were combined, resulting in the precipitation of the corresponding ion paired complex (*i.e.*, tetracycline-AOT). The mixture was centrifuged and the aqueous phase was discarded. The precipitate was washed with cold water to remove unpaired drug and surfactant. After washing, the complex was dissolved in methylene chloride (DCM, Fisher). The tube containing the tetracycline-AOT complex was placed in a ventilation hood, and the DCM was allowed to evaporate. When all DCM was driven off, the dried complex was washed with cold water to remove any remaining salts or free tetracycline. The purified tetracycline-AOT complex was dissolved in water to a final concentration of approximately 0.25 mg/ml, as the tetracycline-AOT complex retains weak aqueous solubility.

Similarly, in other embodiments of the present invention, a hydrophobic ion-paired complex of vancomycin is provided. Briefly, in some embodiments, aqueous stock solutions of vancomycin and AOT were prepared, and equimolar quantities of vancomycin and AOT were combined from the stock solutions by slow dripping followed by thorough mixing. The combined solution turned into a milky white suspension due to the spontaneous precipitation of the vancomycin-HIP complex. This suspension was centrifuged, the supernatant was discarded, and the resulting pellet was washed with cold distilled water. The precipitate was dried overnight in a lyophilizer to form vancomycin-AOT powder.

The solubility properties of HIP-vancomycin were determined, and are shown in Table 1 below.

Table 1

Vancomycin-AOT Solubility Properties

Drug Species	Solvent/Conditions	Solubility
vancomycin	methylene chloride	4.8 mg/ml
vancomycin	water	> 100 mg/ml
vancomycin-AOT	water	0.935 mg.ml
vancomycin	aqueous (*i.e.*, upper phase) (Bligh-Dyer method)	2.49 mg/ml
vancomycin	methylene chloride (*i.e.*, lower phase) (Bligh-Dyer method)	2.33 mg/ml (by mass balance)
vancomycin-AOT	aqueous (*i.e.*, upper phase) (Bligh-Dyer method)	2.47 mg/ml
vancomycin-AOT	methylene chloride (*i.e.*, lower phase) (Bligh-Dyer method)	2.37 mg/ml (by mass balance)
vancomycin-AOT	75% methylene chloride 25 % methanol (complex from a direct partitioning in water, washed twice in cold distilled /deionized water, and dried in a speed-vac)	13 mg/ml
vancomycin-AOT	90% methylene chloride 10 % methanol (complex from a direct partitioning in water, washed twice in cold distilled /deionized water, and dried in a speed-vac)	7 mg/ml

As indicated in Table 1, uncomplexed vancomycin is soluble in water (>100 mg/ml) and has relatively poor solubility in methylene chloride (2.33 mg/ml). Conversely, in some preferred embodiments of the present invention, the AOT-complexed form of vancomycin has reduced aqueous solubility (2.47 mg/ml), while having significant solubility in a 75% methylene chloride/25% methanol mixture (13 mg/ml).

In other embodiments, the present invention provides an HIP-complexed form of the anticancer agent cisplatin. In some embodiments, a cisplatin-AOT complex was formed by first combining a 1:2.2 molar ratio of aqueous solutions of cisplatin (3 mM; Aldrich) and hexafluorophosphate (Sigma; $AgPF_6$). After 2 hours, this mixture was centrifuged to remove precipitated silver chloride. Following the centrifugation, the supernatant (containing the cisplatin) was removed and combined with AOT (Sigma) at a 2:1 molar ratio. After thorough mixing, the solution was allowed to settle, then centrifuged to remove uncomplexed AOT from the solution phase. Following centrifugation, the supernatant was discarded, and the pellet was washed with deionized water, centrifuged again, and the precipitate was dried to yield a white greasy solid consisting of the cisplatin-AOT complex. Determination of the final concentration of the cisplatin-AOT complex was made by dissolving a portion of the dried solid in dimethyl sulfoxide (DMSO) for use in atomic absorption (AA) spectrometry.

In further embodiments, the present invention also provides HIP complexes comprising tacrine. In some embodiments, the present invention provides three different HIP-tacrine complexes. These compounds were synthesized using the linear surfactants sodium dodecyl sulfate, sodium tetradecyl sulfate, and sodium octadecyl sulfate. Separate aqueous stock solutions of tacrine and the linear surfactants were prepared, all at identical molar concentrations. Equal volumes of the drug and surfactant solutions were combined and mixed vigorously, whereupon the ion-paired complexes precipitated out of solution. Reaction mixtures were centrifuged, the supernatant discarded, and the resulting pellet vacuum dried. After drying, the complexes were ground using a mortar and pestle to produce a fine powder and washed with water using a Millipore suction filter system in order to remove any un-paired tacrine and salts from the powdered HIP-complex. Following the washing, the ion-paired complexes were dried again under vacuum.

In yet other embodiments, the present invention also provides HIP-complexes comprising L-phenylephrine. In some embodiments, an aqueous stock solution of L-phenylephrine hydrochloride (Sigma) was prepared, as well as aqueous stock solutions of the surfactants sodium octyl sulfate, sodium dodecyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate (Aldrich), and AOT (Sigma) all in identical molar concentrations. Equal volumes of the drug and surfactant stock solutions were combined to form the aqueous-insoluble ion-paired complexes. To these suspensions an equal volume of the organic solvent methylene chloride (Fisher) was added. The suspension was then

vigorously mixed to insure complete partitioning, centrifuged to separate and isolate the aqueous and organic phases, and the drug concentration in the aqueous and organic phases were measured using UV spectrophotometry at 274 nm (in the aqueous phase) and mass balance (organic phase).

In yet other embodiments, the present invention also provides HIP complexes comprising isoniazid (isonicotinic acid hydrazide). In some embodiments, the HIP-isoniazide complexes are produced using the method of metal-assisted hydrophobic ion pairing, as provided in the present invention, and discussed in detail below. The invention also provides HIP complexes comprising a covalently modified forms of isoniazid (*e.g.*, sodium isoniazid methanesulfonate (IHMS)), using cationic surfactants, also discussed below.

It is not intended that the present invention be limited to any one protocol for HIP complex formation, nor is it intended that the present invention be limited to the use of the surfactants and pharmaceuticals described herein to form HIP complexes. Indeed, other suitable methods and reagents find use with HIP complex formation methods and compositions of the present invention.

II) Metal-Assisted Hydrophobic Ion-Pairing

The HIP process is very adaptable, as it allows one to ion-pair essentially any ionic drug compound and change its partitioning and release properties. HIP is an inexpensive, rapid and reversible process, and no covalent chemical modification of the pharmaceutical occurs. The HIP process is useful in the production of complexes with negatively charged pharmaceuticals using cationic surfactants, or conversely, HIP complexes can be formed comprising positively charged drugs (*e.g.*, tetracycline or vancomycin) and anionic surfactants (*e.g.*, AOT or SDS).

However, there are limitations to the HIP process. The HIP protocol known in the art (*See*, U.S. Patents Nos. 5,770,559 and 5,981,474, herein incorporated by reference) can not form HIP complexes with neutral or zwitterionic molecules. Furthermore, the HIP method requires that surfactants of the opposite charge to the drug be employed.

The present invention provides improved methods for HIP complex formation. In particularly preferred embodiments, the present invention provides metal-assisted hydrophobic ion-pairing (MHIP) methods, which permit the synthesis of HIP complexes using neutral and zwitterionic pharmaceuticals. MHIP involves an initial complexation of

the target pharmaceutical with a metal ion, preferably a multivalent metal ion, before proceeding with the HIP process. The present invention provides various MHIP complexes involving diverse pharmaceuticals, thus demonstrating the wide applicability of this process. In other embodiments, the present invention further provides MHIP complexes with a small neutral molecule and a polyionic zwitterionic compound (a protein).

Forming an HIP Complex with a Neutral Compound

In methods involving the formation of an HIP complex with a neutral molecule having an amine group, that group can be used advantageously, as the group can be protonated prior to HIP complexing in order to generate a charged site to permit amphiphilic ion-pairing. Thus, in these embodiments, the amine group acts as a Lewis base, and thus can act as a ligand to a metal ion. Once the amine is bound, the amine-metal complex can interact with an anionic detergent to form a neutral complex which is significantly soluble in an organic solvent. This approach is useful in cases where the amine is unstable at certain pH values or when the pK_a of the molecule is very low.

For example, isoniazid (isonicotinic acid hydrazide; INH), an anti-tuberculosis drug shown in Figure 3, contains a Lewis base moiety that has a pKa of ~1. Thus, in order to form a traditional HIP complex, a very low (and impractical) pH would be required. Yet, using the MHIP process of the present invention, isoniazid acts as a ligand to a divalent metal, which then forms an HIP complex, as shown in Figure 3. Thus, in some embodiments, isoniazid (2 mmol) was dissolved in 0.5 ml of an aqueous solution of 0.10 M NaCl. Another solution was prepared containing 1 mmol of zinc chloride and 2 mmol of sodium bis(2-ethylhexyl)sulfosuccinate (AOT) in 0.5 ml of distilled water, which was added to the isoniazid solution. The resulting MHIP complex was partitioned into 1 ml of a water immiscible solvent (either 1-octanol or dichloromethane) by addition of the organic solvent, and vortexing the sample followed by centrifugation. The resulting solution contained two clear phases. The relative concentration of isoniazid in the two phases allowed the calculation of the partition coefficient to be made.

The partitioning behavior of the HIP-isoniazid complex, formed using various molar concentrations of drug, zinc and surfactant was examined using water and methylene chloride solvent phases, the results of which are shown below in Table 2.

Table 2

Partitioning Behavior of HIP-Isoniazid (at pH 4.0) Between Water and Methylene Chloride

Molar Ratio Zn^{2+}:drug	Molar Ratio AOT:drug	Molar Ratio Cl^-:drug	*P* Value[#]
0	0	0	0.050
0	0	2.0	0.044
0	1.0	0	0.711
0	2.0	0	0.122
0	2.0	2	0.282
1.0	0	0	0.095
0.5	1.0	0	2.515
0.5	1.0	2.0	1.975
0.5	2.0	0	1.482
0.5	2.0	2.0	2.802
1.0	0	0	0.036
1.0	1.0	0	6.047
1.0	2.0	0	5.383
1.0	1.0	2.0	2.946
1.0	2.0	2.0	3.816

[#] *P* value is the partitioning coefficient, which is calculated from isoniazid concentrations (mg/ml) in the aqueous and organic phases, assuming the extinction coefficient in methylene chloride equals that in water.

As indicated in Table 2, the addition of zinc to the isoniazid solution prior to the HIP reaction resulted in a ten- to fifteen-fold increase in partitioning relative to the absence of zinc, allowing the production of concentrations of approximately 1 mg/ml of the MHIP complex. This compares to maximum concentrations of 0.02-0.04 mg/ml obtainable

without the use of zinc or AOT. Neither zinc nor AOT alone provide enhancement in partitioning to any appreciable degree. Similar results were obtained at pH 6, with five- to six-fold enhancements. Higher pH values were difficult to assess due to insolubility of zinc salts. However, an understanding of the mechanisms involved is not necessary in order to use the present invention.

Additional experiments conducted during the development of the present invention examined the optimal stoichiometry of drug, metal ion, and surfactant molar ratios, and examined partitioning ratios obtained in other organic solvents, as shown below:

Table 3

Partitioning Behavior of HIP-Isoniazid Between Water and Octanol

Component (molar equivalents)			log *P* (octanol/water)
Isoniazid	**$ZnCl_2$**	**AOT**	
1.0	--	--	0.05
1.0	0.5	--	0.10
1.0	--	2.0	0.12
1.0	0.5	1.0	2.5
0.5	1.0	1.0	5.4
0.5	1.0 $MgCl_2$	1.0	<0.1
0.5	1.0 $CaCl_2$	1.0	<0.1

Table 4

Partitioning Behavior of HIP-Isoniazid Between Water and Dichloromethane

Stoichiometry Analysis

Component (molar equivalents)			Relative log *P* (dichloromethane/water)
Isoniazid	$Zn(OTf)_2$	AOT	
1.0	1.0	1.0	1.0
2.0	1.0	1.0	14.7
4.0	1.0	1.0	5.6
8.0	1.0	1.0	1.4

Table 5

Partitioning Behavior of HIP-Isoniazid Between Water and Dichloromethane

Metal-Dependency Study

Component (molar equivalents)			log *P* (dichloromethane/water)
Isoniazid	Metal (1.0 Equivalents)	AOT	
4.0	$CaCl_2$	2.0	0.1
4.0	$ZnCl_2$	2.0	0.77
4.0	$Zn(Otf)_2$	2.0	0.79

In a most preferred embodiment of the MHIP method, the preparation of a metal-assisted hydrophobic ion-paired complex of isoniazid requires the addition of 2 molar equivalents of isoniazid to an aqueous solution containing one equivalent of zinc chloride. To this solution, two equivalents of an anionic detergent (*e.g.*, AOT) are added. The resulting complex is readily partitioned into a water-immiscible solvent, such as 1-octanol. The AOT:metal ratio was not optimized in all of these experiments. However, Tables 2, 3 and 4 indicate that one equivalent of divalent zinc ion interacts with more than one equivalent of drug, and likely best carries two isoniazid molecules. Based on the octanol

partitioning experiments in Table 3, the use of calcium or magnesium in place of zinc resulted in little, if any, increase in partitioning. Only weak effects were observed when calcium was used in place of zinc. Thus, in this case, the successful use of zinc and unsuccessful use of calcium or magnesium in the MHIP reaction was not predicted. However, an understanding of the mechanism(s) is not necessary in order to use the present invention.

Use of MHIP with Polyionic Zwitterions, such as Proteins

Zwitterions, such as proteins, contain multiple charged groups, consisting of both cationic and anionic moieties. The rationale for applying the MHIP process to proteins is that calcium ions are able to bind negatively charged carboxylate groups in proteins. Following the binding of the calcium ion, it is contemplated that the carboxylate moiety is converted from having a single negative charge to one that has an overall charge of +1. The site can now bind, as opposed to repel, an anionic surfactant, such as SDS or AOT. This method to produce MHIP complexes comprising proteins was demonstrated using the protein α-chymotrypsin (CMT). Partitioning properties of MHIP complexes comprising this protein in organic solvents and water as a function of calcium ion concentration was determined, and is shown in Table 6. However, an understanding of the mechanism(s) is not necessary in order to use the present invention.

Table 6

Solubility of α-Chymotrypsin (CMT) in Organic Solvents as a Function of Calcium Ion Concentration

pH	Molar Ratio AOT:CMT	Ca^{2+} Concentration (mM)	Solubility (mg/ml)
Isooctane			
5	25	10	0.310
5	25	20	0.430
5	25	33	0.580
5	25	0 (w/ 50 mM NaCl)	0.004
7	25	0 (w/ 50 mM NaCl)	0.026
7	25	10	0.130
Methylene Chloride			
5	50	0	0.013
5	50	20	0.340

As indicated in Table 6, the enhancement in solubility of MHIP complexes formed in the presence of calcium enhances the organic solubility from five- to one hundred-fold. The inclusion of sodium in the MHIP reaction does not impart improved organic solubility.

In view of these results, it is contemplated that a singly charged carboxylate compound may be manipulated to form an MHIP complex by the addition of one equivalent of a divalent metal to form a metal complex with an overall charge of +1. It is contemplated that this metal complex is then used as an HIP substrate, where exchange of the counter ion with one equivalent of AOT produces an MHIP complex with significant organic solubility. However, an understanding of the mechanism(s) is not necessary in order to use the present invention.

It is contemplated that all of the current advantages and applications of HIP are realized using the MHIP process of the present invention, including the use of MHIP-complexed pharmaceuticals in conjunction with precipitation with a compressed antisolvent (PCA). Moreover, the MHIP complexes appear to have sufficient solubility to allow them to be dissolved in a variety of organic solvents, but are not so hydrophobic that they cannot

be processed using PCA technology to form fine powders and polymeric microspheres. The MHIP method of the present invention expands the range of ionic compounds that can be used with PCA technology. It is also contemplated that the solubility profile of some more hydrophobic compounds is modulated by forming metal complexes. In short, this technology facilitates the application of current HIP/PCA methodologies to be applied to new classes of compounds.

III) Improved Efficacy of Drugs by Hydrophobic Ion Pairing

HIP-complexed drugs have advantageous properties compared to the corresponding non-complexed drug forms. As an HIP-drug complex is lipophilic, it is contemplated that the drug complex is more readily able to translocate across a cell membrane and/or cell wall (*e.g.*, either a mammalian cell membrane or a bacterial cell wall) due to its increased lipophilicity, and thus, more drug is able to accumulate within the target cells of a patient than is possible using the corresponding chloride salt forms of a drug. It is contemplated that this improved biotransport property also extends to microorganisms (*e.g.*, bacteria), in the case where the drug is an antimicrobial. This provides important advantages, as the potency of a given drug concentration is improved, and lower concentrations or fewer doses of a toxic drug are required in order to achieve a desired therapeutic response. The use of lower concentrations and/or fewer dosages of some drugs is an advantageous embodiment of the present invention, particularly for drugs that exhibit significant toxicity when administered to patients.

The present invention provides compositions and methods for improving the efficacy of pharmaceuticals. In particularly preferred embodiments, the present invention provides compositions and methods to modify pharmaceutical agents by forming an HIP complex to produce a pharmaceutical compound with improved efficacy. Although an understanding of the mechanisms is not necessary in order to practice the present invention, it is believed that improved drug efficacy is the result of enhanced transport capability produced by increasing the biomolecule's lipophilicity.

Improved Efficacy of Hydrophobic Ion Paired Tetracycline

In some embodiments, the present invention provides tetracycline-AOT complexes in which the efficacy (*i.e.*, antimicrobial activity) of the tetracycline is improved, as compared to the activity of non-modified tetracycline (*e.g.*, as determined in an *E. coli* growth rate assay). The analysis of *E. coli* culture growth rates in response to free tetracycline and a tetracycline-AOT complex is described in detail in Example 9. The tetracycline-AOT complex used in the experiments was produced as described in Example 1.

The improved ability of tetracycline-AOT to suppress *E. coli* growth rate compared to non-complexed tetracycline is illustrated in Figure 7. This experiment was conducted using 0.6 μg/ml concentrations of tetracycline and tetracycline-AOT complex. As shown in this Figure, the ion-paired tetracycline inhibited the growth rate of the *E. coli* culture 45% more effectively than the free tetracycline. In parallel, a bacterial culture treated with only the AOT moiety demonstrated that the growth inhibition was not caused by the AOT alone. Almost immediately following the addition of the tetracycline-AOT complex, a reduction in growth rate of the *E. coli* culture was observed.

Improved Efficacy of Cisplatin by Hydrophobic Ion Pairing

The use of cisplatin, like other chemotherapy drugs, is associated with significant toxicity, in particular, neurotoxicity and nephrotoxicity. Thus, the clinical application of cisplatin is limited to low-dose levels. The present invention provides methods that reduce the aqueous solubility and increase the lipophilicity of cisplatin, increasing the intracellular delivery rate of the drug, thereby improving the efficacy of the drug. Thus, the concentration of drug required to produce a therapeutic benefit can be lowered or the number of required doses reduced, thereby minimizing toxic effects on a patient who receives the drug.

In some embodiments of the present invention, a more lipophilic form of cisplatin is produced through hydrophobic ion pairing, resulting in enhanced intracellular bioavailability of the HIP-cisplatin complex relative to a non-complexed form of cisplatin. In addition, similar or higher therapeutic effects can be achieved using the ion-paired form compared to the parent form at a given dose level, reducing or avoiding the toxic effects of cisplatin through use of an ion-paired form of the drug.

One embodiment for the synthesis of a cisplatin-AOT complex is described in Example 3. The intracellular availability and cytotoxic efficacy of the cisplatin-AOT complex were compared to those activities of non-complexed cisplatin in a tissue culture model system using Chinese hamster ovary (CHO) cells. The intracellular concentrations of platinum were measured following treatment of cultured mammalian cells with cisplatin or a cisplatin-AOT complex, and the cytotoxic activities of the cisplatin forms were compared using a cell culture cytotoxicity assay.

To measure the intracellular accumulation (*i.e.*, bioavailability) of platinum following treatment with cisplatin or cisplatin-AOT, CHO cells were plated into culture dishes and exposed to either cisplatin or cisplatin-AOT complex for one hour. Following this exposure, the intracellular levels of platinum were quantitated by harvesting the cells, normalizing for cell numbers, and analyzing by atomic absorption (AA) spectrometry. Two similar experiments were conducted, both yielding similar results, as described in Example 10 and Figure 9.

In brief, the cell cultures exposed to cisplatin-AOT showed intracellular platinum concentration of $1x10^{-5}$ μM, while the culture exposed to 50 μM cisplatin-AOT showed an intracellular platinum concentration at least four-fold higher than cultures exposed to the parent cisplatin. Thus, it is contemplated that this improved ability of cisplatin-AOT to accumulate within the cells results in greater cytotoxic activity of a given dose of cisplatin-AOT compared to non-complexed cisplatin.

To assess the cytotoxic activities of cisplatin and cisplatin-AOT, a plating efficiency/cell survival assay was used. In this assay, CHO cell cultures were exposed to cisplatin or cisplatin-AOT at a range of concentrations for one hour. Following this exposure, the cells were harvested, counted, and replated at a final density of 100-500 cells per dish for each drug and exposure concentration. These cultures were allowed to grow an additional 7 days, after which time the percentage of originally plated cells which survived and grew on the plates was determined. The plating efficiency data for these cisplatin and cisplatin-AOT exposed cultures is shown in Figure 10. Based on these plating efficiency assays, the LD_{50} (50% of lethal dose) of cisplatin in this experimental system was approximately five-fold higher than the LD_{50} for cisplatin-AOT. Furthermore, the plating efficiency of cultures treated with cisplatin-AOT was consistently and significantly lower than cultures treated with cisplatin at practically the full range of drug concentrations.

From these data, it is concluded that the cisplatin-AOT drug complex has greater cytotoxic activity than the parent cisplatin.

These experiments demonstrate that HIP-complexed pharmaceuticals (*e.g.*, tetracycline-AOT and cisplatin-AOT) are more efficacious than the non-complexed forms of the pharmaceuticals. Indeed, the HIP complexes of the present invention demonstrate advantageous activities at concentrations where the parent chloride salt has little or no effect. In some preferred embodiments, these complexes maintain their integrity in bacterial or mammalian cell culture media, allowing them to penetrate more easily into cells. Thus, the present invention provides methods and compositions for the development of pharmaceutical preparations with increased potency and efficacy. It is contemplated that the present invention will find use in the efficacious administration of commonly used (as well as less commonly used) drugs at concentrations lower than are currently used. In addition, the toxicity of the pharmaceutical compound is minimized.

Various surfactants have been utilized for their ability to enhance the permeability of drugs across biological membranes. However, in most cases reported to date, the surfactant is typically present at concentrations high enough to cause higher order aggregates and/or micelles, which in turn allows for strong interactions with the drug, thereby reducing drug activity. The development of the present invention has overcome these obstacles, to provide compounds useful for the improved treatment of patients.

IV) Use of Covalently-Modified Drugs in Hydrophobic Ion Pairing

The HIP process is very adaptable, as it allows one to ion-pair essentially any ionic drug compound and change its partitioning, efficacy and release properties. In some embodiments, the HIP process is used to form HIP complexes with negatively charged pharmaceuticals using cationic surfactants, or conversely, HIP complexes are formed comprising positively charged drugs and anionic surfactants.

However, the HIP process known in the art has a limitation, as it cannot form HIP complexes comprising neutral molecules. For example, isoniazid (isonicotinic acid hydrazide) is a neutral molecule at physiological pH, and is unable to form a traditional HIP complex. However, the present invention provides methods for the HIP complexing of isoniazid.

For example, a prodrug approach was used to introduce a negatively charged group onto the neutral isoniazid molecule, to yield sodium isoniazid methanesulfonate (IHMS) (*See*, U.S. Patent No. 2,759,944, herein incorporated by reference), as described in detail in Example 8. The IHMS species has been demonstrated to retain anti-tuberculosis activity *in vivo* and *in vitro*. As described by the present invention involving the synthesis of the IHMS species permits ion-pair complex formation. Following synthesis of the covalently-modified IHMS, a number of HIP complexes were formed using different cationic surfactants, resulting in the formation of complexes that were relatively water-insoluble and organic-soluble. In contrast, no surfactant facilitated organic phase transfer of non-modified isoniazid. These results demonstrate the importance of the added negative charge on the isoniazid derivative in the formation of the HIP species.

During the development of the present invention, numerous cationic surfactants were used to produce IHMS-HIP complexes, although three were chosen for further study. These three are tetraheptylammonium bromide, tetrapentylammonium bromide and tetraoctylammonuum bromide (Fluka). Other cationic lipids used to produce HIP complexes of IHMS using this method included:

trimethylalkylammonium bromide,
tetraalkylammonium bromide,
1,2-diacyl-3-trimethylammonium-propane,
dodecyltrimethylammonium bromide,
tetradecyltrimethylammonium bromide,
hexadecyltrimethylammonium bromide,
tetraethylammonium bromide,
tetrabutylammonium bromide,
1,2-dimyristoyl-3-trimethylammonium-propane,
1,2-dipalmitoyl-3-trimethylammonium-propane,
1,2-distearoyl-3-trimethylammonium-propane, and
1,2-dioleoyl-3-trimethylammonium-propane.

Following the formation of the HIP complexes, the complexes were purified and dried as described in Example 8. As indicated above, three of HIP complexes were chosen for further study. The water/dichloromethane partition coefficients for IHMS-tetrapentylammonium, IHMS-tetraheptylammonium and IHMS-tetraoctylammonium

HIP complexes were determined. The results of this analysis are displayed in Figure 4, as log *P*, where:

$$\log P = \log \left(\frac{[drug]_{organic\ phase}}{[drug]_{aqueous\ phase}} \right)$$

These partitioning coefficients were calculated as described in Example 8, and the values are shown in Figure 4. As indicated by the graph in this Figure, the parent isoniazid and the covalently modified IHMS have negative log P values, indicating a preference for solubility in aqueous solution. However, HIP-complexes of the IHMS molecule all show preferential solubility in the organic phase, with the IHMS-tetraoctylammonium form showing the highest preference for solvation in the organic solvent. In contrast, no surfactant facilitated organic phase transfer of non-modified isoniazid.

Thus, the covalent modification of drugs is useful in the production of HIP complexes comprising drugs that can not be ion-paired using HIP techniques know in the art. However, it is not intended that the present invention be limited to the covalent modification of isoniazid, as it is contemplated that covalent modification is applicable to numerous pharmaceuticals, so as to make such pharmaceuticals suitable for use in the HIP process. Furthermore, it is not intended that the present invention be limited to the methyl sulfonate reaction provided, as numerous other covalent modification reactions are equally effective at adding charged groups to molecules. It is also not intended that the present invention be limited to the surfactants used to produce the HIP complexes, described herein as numerous cationic surfactants find use with the invention.

V) Use of Hydrophobic Ion Paired Drugs for Controlled Drug Release

In some preferred embodiments, HIP-complexed drugs act as delayed-release systems, as the slow dissociation (*i.e.*, reverse ion pairing) of a water-insoluble HIP-drug complex slowly releases free drug (*i.e.*, water-soluble drug) into the biological system. In some embodiments, such an HIP complex are viewed as a "depot delivery formulation." In particularly preferred embodiments, the present invention provides "controlled-release" or "delayed-release" drug compositions comprising HIP-complexed pharmaceuticals, in which delivery ranges from hours to days rather than the short term delivery of transitional administration methods (*i.e.*, minutes).

In order to demonstrate the controlled release properties of HIP complexes, the kinetics of vancomycin-AOT reverse ion pairing were studied *in vitro* using PBS as the exchange buffer. Concentrations of vancomycin were measured spectrophotometrically. The vancomycin-AOT complex was synthesized as described in Example 2.

The release profile resulting from the reverse ion-pairing of vancomycin-AOT is shown in Figures 12 and 13. Figure 12 provides the cumulative percentage of vancomycin released into a PBS buffer (*in vitro*) versus an arithmetic time scale, while Figure 13 depicts this same information on an exponential time scale. These graphs demonstrate that the half-life of the vancomycin-AOT complex in PBS is on the order of a few days (2 or 3 days). This slow rate of reverse ion pairing (with a half-life on the order of 24-72 hours *in vitro* in PBS) is unexpected, as it is a widely held belief in the art that HIP complexes dissociate quickly, on the order of minutes, in isotonic buffers.

Although an understanding of the mechanism(s) is not necessary in order to use the present invention, it is believed that the rate of HIP complex dissociation is a function of the nature of the amphiphilic surfactant in the complex. That is, the more lipophilic in nature is the surfactant, the more stable the HIP complex is likely to be, and thus, the rate of reverse ion-pairing in an ionic aqueous environment of that complex is likely to be slower that for a complex that incorporates a less lipophilic surfactant. One method of monitoring the "strength" of the hydrophobic ion-pairing involves measuring the partitioning coefficient (*P*), which measures the equilibrium of drug concentration in the organic and aqueous phases in a solubility ratio, where:

$$P = \frac{[organic]}{[aqueous]}$$

A study of the partitioning behavior of a series of HIP complexes formed with L-phenylephrine was undertaken. The HIP complexes incorporated a variety of surfactants. The partitioning coefficients calculated for these complexes used a methylene chloride organic phase. Figure 2 shows the effect of surfactant characteristics on the hydrophobicity of the ion-paired complex. The hydrophobicity of each surfactant used in the present study was quantified using the hydrophilic-lypophilic balance number (the HLB number) for each

surfactant, where the HLB number is determined by correlating the surfactant structure with its effectiveness as an emulsifier.

$$HLB = \frac{\%\ of\ hydrophilic\ group}{5}$$

As indicated in Figure 2, the partitioning efficiency of the L-phenylephrine ion-paired complexes into the organic phase decreases as the surfactant HLB number increases (*i.e.*, as the hydrophilic nature of the surfactant increases).

Although an understanding of the mechanism(s) is not necessary in order to use the present invention, it is believed that the Ion-paired surfactant-drug complexes disassociate because a lower chemical potential can be achieved by pairing with other ions in solution. The fluids of the human body contain salt ions, such as sodium, which may generate this behavior. As a result, it is contemplated that an HIP-complexed drug is slowly released into solution beyond the ion-paired drug complex's solubility limit as the drug is freed from the complex through an ion exchange process. To determine the influence of surfactant characteristic over this exchange rate, the dissolution profile of several drug-surfactant complexes in continuously stirred PBS was measured. These HIP complexes all incorporated tacrine, and alternatively used the surfactants sodium dodecyl sulfate, sodium tetradecyl sulfate, or sodium octadecyl sulfate. These HIP complexes were synthesized as described in Example 5.

To study the dissociation of these complexes, a sample of the ion-paired tacrine complex was placed in a continuously stirred flask containing 0.1 M NaCl and 20 mM phosphate buffer, pH 7.4 (*i.e.*, PBS). At designated times, samples were collected, and the concentration of tacrine at each time point was measured using UV-spectrophotometry at 324 nm.

The results of this dissolution sampling are shown in Figure 1. As indicated in this Figure, the dissolution profiles using the various surfactant HIP complexes showed a range of dissociation rates. The dissociation rate of some of the complexes were slower than others, with the HIP complexes formed from the octadecyl sulfate surfactant the longest lived.

Again, although an understanding of the mechanism(s) is not necessary in order to use the present invention, the dissolution characteristics of the various tacrine HIP complexes in salt solutions can be modelled and partially predicted assuming a biphasic behavior requiring two independent 1st order rate constants. The tacrine-surfactant ion-paired complex rapidly dissolved into the aqueous ionic solution up to the complex's solubility limit. Once in solution, the presence of an aqueous salt begins the reverse ion exchange process eventually obtaining a chemical equilibrium between the dissolved drug and the dissolved drug-surfactant complex. As a result, the dissolution mechanism has two kinetic rate constants. The first rate constant governs the complex's phase change from a solid to liquid and can be derived from de-ionized water release values modeled by a single, non-zero 1st order rate constant, while the other rate constant governs the ion exchange rate, and taking into account the salting in effects. Figure 1 shows the good agreement achieved between the predicted release profile and the experimental data. Table 2 shows the water and PBS solubilities of the ion-paired complexes, in addition to the HLB numbers for the corresponding surfactants.

Table 7

Solubilities of Ion-Paired Tacrine Complexes

Surfactant	HLB number	Complex Solubility (μg/ml)	
		PBS	DI-water
sodium dodecyl sulfate	2.8	16.5	16.0
sodium tetradecyl sulfate	2.4	5.6	4.4
sodium octadecyl sulfate	2.0	2.3	1.0

Thus, it is contemplated that control of the ion exchange process through surfactant characteristics provides control over drug release into solution environments with free ions at concentrations typical of biological systems (*e.g.*, the human body). As indicated in Figure 2 and Table 7, decreasing the hydrophobic characteristic of the pairing surfactant increases the quantity of drug dissolved or becoming available for circulation within salt solutions compared to surfactants that exhibit higher hydrophobicity. However, it is

recognized that the actual time duration for release depends upon the type of surfactant used and the circulation rate experienced at the administration site.

In contrast to the widely held belief in the art that HIP-complexed pharmaceutical compositions are of little value in improving pharmaceutical efficacy, the present invention provides numerous advantages over traditional drug delivery methods. The ability of the ion-paired complex to remain intact for many minutes or even hours or days under biological conditions was an unexpected finding that is in direct contrast with the common belief that these HIP complexes dissociate in biological media relatively quickly. Furthermore, the observation that increased hydrophobicity of the HIP-complex allows prolonged release of the complexed pharmaceutical was both a surprising and advantageous observation.

Although vancomycin, tetracycline and isoniazid were used during the development of HIP complexes of the present invention, it is not intended that the present invention be limited to these antimicrobials or any subgroup of pharmaceuticals. Indeed, numerous other antimicrobials are suitable for use with the present invention. In addition, the present invention encompasses HIP complexes comprising various other bioactive compounds such as growth factors, hormones, cytokines, anti-inflammatories, etc., in order to provide improved therapeutic benefits with these agents. The prolonged release of antimicrobials, particularly at sites of infection or wounds that are refractory to treatment using traditional methods, provides many significant advantages and facilitates the improvement in the quality of life for affected individuals. In some embodiments, the present invention facilitates at-home care, reducing the necessity for hospitalization and the associated risks of nosocomial infection. Thus, the delivery of antimicrobials in HIP complexes to wound sites and surgical sites provides advantages over current antimicrobial treatment regimens.

VI) Delivery of Hydrophobic Ion Paired Drugs Using Poloxamer Gels

In one embodiment, the present invention provides approaches for the controlling the rate of release of a parenterally administered drug (*e.g.*, an HIP-complexed pharmaceutical). In one particularly preferred embodiment, the drug is entrapped within a matrix, gel or bead, from which the drug slowly diffuses following administration or implantation. Alternatively, the drug is released from such a matrix via the slow biodegradation of the matrix material.

Examples of products which finds use with the present invention are poloxamer gels (block co-polymers of poly[oxyethylene] and poly[oxypropylene]), sold under the brand name PLURONICS (*See*, U.S. Pat. Nos. 4,100,271 and 5,457,092, both of which are herein incorporated by reference). These polymers are water soluble, and when dissolved in water certain members of the poloxamer family (*e.g.*, poloxamer 407, PLURONIC F-127) exhibit the unusual behavior of "reverse thermal gelation," in which the poloxamer solution is liquid at low temperatures (such as room temperature), but has gel consistency at higher temperatures (*e.g.*, 37°C). This unusual gelation property of poloxamer facilitates the synthesis of suitable poloxamer-drug mixtures, as well as simplifying administration to a patient. The poloxamer gels, and specifically poloxamer 407, also have the advantageous characteristics of providing a microenvironment that is compatible with protein stability, and preserves protein activity.

In some preferred embodiments, the water-insoluble HIP-pharmaceutical complex is suspended in a suitable gel, or a liquid solution that will form a gel (*e.g.*, poloxamer 407). In a most preferred embodiment, the application of a poloxamer/HIP-antimicrobial composition to a wound provides a reservoir drug delivery system capable of delivering an active level of antimicrobial over a clinically-useful period of many hours to days (*e.g.*, up to 14 days). Although an understanding of the mechanism is not necessary in order to practice the present invention, the water-insoluble HIP-antimicrobial complex undergoes a natural reverse ion pairing when exposed to physiological fluids. This reverse ion-pairing releases the soluble form of the antimicrobial to the surrounding tissue at a rate that is sufficiently slow to provide a slow and controlled release. Furthermore, in some embodiments, the HIP-antimicrobial diffuses from the matrix material, or is released from the matrix material as the matrix undergoes degradation. In addition, the hydrophobic form of antimicrobial(s) penetrates the surrounding tissue more effectively than the corresponding hydrophilic salt form of the antimicrobial.

In some embodiments, poloxamer gels provide suitable gel matrices for HIP-pharmaceutical complexes. However, it is not intended that the invention be limited to the use of poloxamer gels, as other appropriate gel compositions also find use with the present invention.

In one embodiment, the present invention provides a poloxamer gel containing a vancomycin-AOT ion-paired complex. Synthesis of the vancomycin-AOT is described in Example 2, and construction of the poloxamer-vancomycin-AOT composition is described

in Example 12. Briefly, solutions of poloxamer 407 (22% w/w in water) containing 10 mg/ml (adjusted weight) vancomycin or vancomycin-AOT were prepared. To compare the systemic concentrations of vancomycin following the subcutaneous delivery of vancomycin-poloxamer or the vancomycin-AOT-poloxamer formulations to mice, plasma levels of the antimicrobial were assayed at time points following subcutaneous administration of the poloxamer.

Thirty-six mice were divided into three groups of 12 mice each. These groups were treated as follows:

Group I: given a subcutaneous injection of non-complexed vancomycin in a poloxamer solution into the mouse flank fat pad;

Group II: given a subcutaneous injection of HIP-complexed vancomycin suspended in DI water;

Group III: given a subcutaneous injection of HIP-complexed vancomycin in poloxamer solution.

The dose level of vancomycin delivered to each mouse in the poloxamer gel was 100 mg vancomycin per kg of mouse weight.

Three animals from each group were sacrificed at days 1, 2, 4 and 8. The vancomycin levels in the plasma were assayed using a TDx clinical analyzer (Abbott Laboratories). The TDx instrument detects fluorescence polarization of a fluorescently-tagged, polyclonal antibody specific for the analyte in question (*i.e.*, an anti-vancomycin antibody). The larger the signal, the greater the concentration of vancomycin. The results of this assay are provided in Figure 11. As indicated in this Figure, poloxamer delivery of HIP-complexed vancomycin provides a sustained plasma concentration of vancomycin over at least eight days following injection of the poloxamer matrix.

Thus, in some embodiments, the gel/HIP-antimicrobial composition of the present invention find particular use in the treatment and prevention of infections in which controlled drug release is desirable. For example, the present invention provides improved methods and compositions for treating diabetic patients presenting with ulcers of the extremities (*e.g.*, foot and hand ulcers). It is also contemplated that the compositions and methods of the present invention will find use in the prevention and/or treatment of infections associated with surgical incision sites. However, it is not intended that present invention be limited to treating or preventing any particular type of patient, disease or

wound. Indeed, the present invention finds use in numerous settings and treatment regimens.

In some embodiments, the present invention provides compositions and methods for the prolonged delivery of antimicrobial(s) to a site to be treated (*e.g.*, a lesion) over a period of days. In preferred embodiments, the compositions of the present invention comprise at least one modified antimicrobial of choice suspended in a suitable gel. In some embodiments, the antimicrobial modification results in a form of the antimicrobial that is less water-soluble than the unmodified form. This gel/antimicrobial suspension is applied to a site to be treated (*e.g.*, a diabetic ulcer), or on, in or around a surgical incision site following a surgical procedure, or any other type of wound. However, it is not intended that the present invention be limited to any particular type of lesion, wound, or treatment site. Indeed, the present invention finds use in the treatment of any appropriate pathological process, including but not limited to wounds, ulcers, surgical incisions, abrasions, burns, etc., present either on the external body sites (*e.g.* skin and mucous membranes) or internal sites (*e.g.*, within organs, cavities, vessels, etc.). Thus, the present invention provides methods and compositions that are suitable for the treatment in a wide variety of settings.

EXPERIMENTAL

The following Examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

As used herein, the following scientific abbreviations/notations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); MW (molecular weight); °C (degrees Centigrade); OD (optical density); EDTA (ethylenediamine-tetracetic acid); EGTA (ethyleneglycol-bis-(β-aminoethyl ether)); SDS (sodium dodecyl sulfate); PAGE (polyacrylamide gel electrophoresis); UV (ultraviolet); bp (base pair); kb, kbp or Kb (kilobase pairs); μg/ml (microgram per milliliter); mm (millimeter); x g (times gravity); HPLC (high pressure liquid chromatography); DDT (dithiothreitol); PMSF (phenylmethylsulfonyl fluoride); SDS (sodium dodecyl sulfate); PAGE (polyacrylamide gel

electrophoresis), s and sec (seconds), m and min (minutes), h and hr (hours), w/w (weight to weight measure), w/v (weight to volume measure), v/v (volume to volume measure).

Where manufacturers are indicated, the following abbreviations apply: Aldrich (Sigma-Aldrich Co., Milwaukee, WI), BASF (BASF Corp., Mt. Olive, NJ); Becton Dickinson/BBL (Becton Dickinson-BD Biosciences, Sparks, MD); Life Science Products (Life Science Products, Inc., Denver, CO); Sigma (Sigma Chemical Co., St. Louis, MO); Fisher (Fisher Scientific, Pittsburgh, PA); Fluka (Fluka Chemika-BioChemika, Switzerland); Gibco/BRL/Life Technologies (GIBCO BRL Life Technologies, Gaithersburg, MD); and Scientific Adsorbant (Scientific Adsorbant, Inc., Atlanta, GA)

EXAMPLE 1

Synthesis of a Hydrophobic Ion Paired Complex with Tetracycline

This Example describes the formation of a HIP-tetracycline complex. To synthesize a hydrophobic ion-paired tetracycline complex, tetracycline hydrochloride (Sigma) was dissolved in water at a concentration of 36.8 mg/ml. Similarly, the anionic surfactant dioctylsulfosuccinate sodium salt, Aerosol OT (AOT; Sigma) was dissolved in water to a concentration of 14.0 mg/ml. As this concentration of AOT has poor solubility in water, it was necessary to gently heat the AOT solution until all of the AOT was dissolved. A 1.0 ml volume of the tetracycline solution was slowly added dropwise, to an equal volume of the AOT solution. The ion paired complex of tetracycline and AOT precipitated from the solution, forming a yellow, greasy solid.

The mixture was centrifuged for 10 minutes at 4000 rpm, and the supernatant was removed and discarded. The precipitate was washed 3 times with cold water to remove unpaired drug and surfactant. After washing with cold water, the complex was dissolved in 2 ml of methylene chloride (DCM, Fisher). The tube containing the tetracycline-AOT complex was placed in a ventilation hood, and the DCM was allowed to evaporate. When all DCM was driven off, the dried complex was washed again with cold water to remove any remaining salts or free tetracycline. The purified tetracycline-AOT complex retained poor aqueous solubility, and thus was dissolved in water to a final concentration of approximately 0.25 mg/ml.

EXAMPLE 2

Synthesis of a Hydrophobic Ion Paired Complex with Vancomycin

This Example describes the formation of a hydrophobic ion-paired vancomycin complex. An aqueous stock solution of vancomycin HCl (MW 1486, 16.2 mM) was prepared. Concentration of the vancomycin was confirmed by UV absorbance measurement, where the extinction coefficient is 4 $[(mg/ml)(cm)^{-1}]$ measured at 282 nm. An aqueous stock solution of AOT was also prepared (MW 444.55; 29 mM; 1.29 g AOT in 100 ml water).

A volume of 15 ml of the vancomycin stock solution (equivalent to 0.243 mmols) was added to a 50 ml Falcon polypropylene conical tube. To this, 8.38 ml of AOT stock solution (equivalent to 0.243 mmols) was added by slowly dripping with a transfer pipet, and mixed by slow swirling. The brownish-colored vancomycin solution turned milky white due to spontaneous precipitation of the vancomycin-AOT complex following addition of the AOT.

Following precipitation, the tubes were spun at room temperature for 10 min at 4000 x g. The supernatant was discarded, and the precipitated pellet washed twice in 5 ml cold distilled water with brief vortexing (30 sec on a vortex setting of 5), centrifuged for 5 min at 4000 x g, and the supernatant was discarded. The precipitate was dried overnight under nitrogen to form an HIP-drug powder.

The solubility properties of HIP-vancomycin were determined, and are shown in Table 1. From this Table, it can be seen that uncomplexed vancomycin is soluble in water (>100 mg/ml) and has relatively poor solubility in methylene chloride (2.33 mg/ml). Conversely, the AOT-complexed form of vancomycin has reduced aqueous solubility (2.47 mg/ml), while having significant solubility in a 75% methylene chloride/25% methanol mixture (13 mg/ml).

EXAMPLE 3

Synthesis of a Hydrophobic Ion-Paired Complex with Cisplatin

This Example describes the formation of a cisplatin-HIP complex. Cisplatin (Aldrich; cis-diamminedichloroplatinum (II); cisplatinum diammine chloride) and hexafluorophosphate (Sigma; $AgPF_6$) were combined in a 1:2.2 molar ratio in aqueous solution with a cisplatin concentration of 3 mM. Two hours later, the mixture was spun twice at 1000 x g for 5 minutes to remove precipitated silver chloride.

Following centrifugation, the supernatant was collected and treated with 30 mM AOT surfactant (Sigma) at a 2:1 molar ratio with the cisplatin solution. After thorough mixing, the solution was allowed to settle for 2 hours at room temperature, then centrifuged at 1000 x g for 5 minutes. This centrifugation allowed the removal of uncomplexed AOT from the solution phase. Following the centrifugation, the supernatant was discarded, and the pellet was washed three times with deionized water and centrifuged again as above. Following the wash steps, the precipitate was dried in a lyophilizing centrifuge to yield a white greasy solid consisting of the cisplatin-AOT complex. Determination of the final concentration of the cisplatin-AOT complex was made by dissolving a portion of the dried solid in dimethyl sulfoxide (DMSO) for use in atomic absorption (AA) spectrometry.

EXAMPLE 4

Synthesis of Hydrophobic Ion-Paired Complexes with Tacrine

This Example describes the synthesis of HIP-complexes incorporating tacrine. Three different HIP-tacrine complexes were synthesized using the linear surfactants sodium dodecyl sulfate, sodium tetradecyl sulfate, and sodium octadecyl sulfate.

Aqueous stock solutions of 15 mg/ml tacrine and equimolar aqueous stock solutions of each of the three surfactants were prepared. Occasionally, the processing temperature was increased to facilitate surfactant dissolution into water. Equal volumes of the drug and surfactant solutions were combined and mixed vigorously for 5 minutes, whereupon the ion-paired complexes precipitated out of solution. Reaction mixtures were centrifuged at 2300 rpm for 25 minutes, and the supernatant was discarded. The resulting pellet was vacuum dried. After drying, the complexes were ground using a mortar and pestle to produce a fine powder and washed using a Millipore suction filter system and 150-ml of de-ionized water. Washing removed any un-paired tacrine and salts from the powdered HIP-complex. Following the washing, the ion-paired complexes were dried again under vacuum.

EXAMPLE 5

Measurement of the Dissolution Profiles of Hydrophobic Ion Paired Complexes with Tacrine

This Example describes the dissolution properties of HIP complexes of tacrine in an aqueous ionic solution. The following procedure was repeated for all three surfactant-drug complexes (*i.e.*, HIP complexes containing tacrine, and either sodium dodecyl sulfate, sodium tetradecyl sulfate, or sodium octadecyl sulfate).

A 20 mg sample of the ion-paired tacrine complex was placed in a continuously stirred, tapered flask containing 50 ml of PBS. At designated times, 2.5 ml samples were collected using 0.2 mm pore size syringe filters, and then replaced with fresh 2.5 ml solution. The fresh solution was flushed back through the syringe filter into the flask, thereby limiting the amount of solid sample lost during this procedure. The concentration of tacrine at each time point was measured using UV-spectrophotometry at 324 nm.

The results of this dissolution sampling are shown in Figure 1. As indicated in this Figure, the dissolution profiles using the various surfactant HIP complexes showed a range of dissociation rates. Surprisingly, the dissociation rate of some of the complexes were significantly slower than others, with some of the HIP complexes (*i.e.*, the tactine-octadecyl sulfate complex) were long lived.

EXAMPLE 6

Synthesis of a Hydrophobic Ion-Paired Complex with L-phenylephrine

This Example describes the formation of HIP-complexes using L-phenylephrine and a series of surfactants.

An aqueous stock solution of 2.0 mg/ml L-phenylephrine hydrochloride (Sigma) was prepared. In addition, aqueous stock solutions of the surfactants sodium octyl sulfate, sodium dodecyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate (Aldrich) and sodium dioctyl sulfosuccinate (AOT; Sigma) were prepared at molarities equal to that of the stock drug solution. To form the HIP complexes, equal volumes of the drug and surfactant stock solutions were combined, resulting in the spontaneous formation and precipitation of the ion-paired complexes. An equal volume of the organic solvent methylene chloride (Fisher) was added to these suspensions. The mixture was then vigorously shaken for 3 minutes and transferred to a rotator for continued mixing at 15 Hz for 1 hour to insure complete partitioning. In order to measure the drug content remaining in the aqueous

phase, the solutions were centrifuged at 2300 rpm for 30 minutes. After the phases were successfully separated, the drug concentration in the aqueous phase was measured using UV spectrophotometry at 274 nm. The drug concentration in the organic phase was obtained through a mass balance.

The partitioning behavior of these L-phenylephrine HIP complexes was quantitated; the results are shown in Figure 2. The partitioning number, *P*, is a ratio of the HIP-tacrine in the organic phase versus the aqueous phase, and the hydrophilic-lypophilic balance number (HLB) is a measure of the hydrophilic nature of the surfactant, where the greater the HLB number, the more hydrophilic the surfactant. As indicated in Figure 2, the partitioning efficiency of the L-phenylephrine HIP complex into the organic phase decreased as the surfactant HLB number increased.

EXAMPLE 7

Synthesis of a Hydrophobic Ion Paired Complex with Isoniazid Using Metal-Assisted Hydrophobic Ion Pairing

This Example describes the formation of an isoniazid HIP-complex using metal-assisted hydrophobic ion pairing. Isoniazid (2 mmol) was dissolved in 0.5 ml of an aqueous solution of 0.10 M NaCl. Another solution was prepared containing 1 mmol of zinc chloride and 2 mmol of sodium bis(2-ethylhexyl)sulfosuccinate (AOT) in 0.5 ml of distilled water, which was added to the isoniazid solution. The resulting MHIP complex was partitioned into 1 ml of a water immiscible solvent (either 1-octanol or dichloromethane) by addition of the organic solvent, vortexing the sample for 30 seconds, and then centrifuging at 5000 rpm for 5 minutes. The resulting solution contained two clear phases. The relative concentration of isoniazid in the two phases allowed the calculation of the partition coefficient to be made. If there was an appreciable emulsion-type interface after centrifugation, addition of more NaCl and a repeat of the vortexing and centrifugation steps produced a clear interface.

EXAMPLE 8

Synthesis of a Hydrophobic Ion Paired Complex with a Covalently Modified Isoniazid Species

This Example describes the chemical synthesis of a covalently modified form of isoniazid, followed by formation of HIP-isoniazid complexes using cationic surfactants.

Isoniazid (isonicotinic acid hydrazide; INH) is a neutral pharmacophore that is unable to form HIP complexes under physiological conditions using HIP methods known in the art. A prodrug approach was used to introduce a negatively charged group onto the neutral molecule, to yield sodium isoniazid methanesulfonate (IHMS) (*See*, Figure 3). The IHMS species has been demonstrated to have anti-tuberculosis activity *in vivo* and *in vitro* (Kitamoto *et al.*, *Japanese Journal of Tuberculosis*, 1:92 [1953]; and Orlowski *et al.*, *Arzneim.-Forsch.*, 26:409 [1976]). As demonstrated herein, the synthesis of the IHMS species permits ion-pair complex formation. A number of HIP complexes were formed using different cationic surfactants, resulting in relatively water insoluble and organic soluble species. In contrast, no surfactant facilitated organic phase transfer of non-modified isoniazid (data not shown). These results demonstrate the importance of the added negative charge on the isoniazid derivative in the formation of the HIP species.

The neutral isoniazid (Sigma) was used as the starting material in the synthesis of IHMS. This chemical synthesis is described in U.S. Patent No. 2,759,944 (herein incorporated by reference). An aqueous solution of sodium bisulfite (100 ml, 52 g [0.5 mol] sodium bisulfite) was added to an equimolar mixture of isoniazid (68.5 g, 0.5 mol) in formaldehyde (39%, 38 ml). The mixture was heated to 100°C for 8 hours and was then evaporated under reduced pressure to give a yellow solid. Recrystallization from an ethanol water mixture yielded white, solid sodium IHMS. The derivative was characterized by both ^{1}H and ^{13}C NMR spectra (data not shown).

To form IHMS hydrophobic ion-paired complexes, a 1.0 mM aqueous solution of sodium IHMS was mixed with 2.1 volumes of methanol. One volume of 1.0 mM cationic surfactant solution in dichloromethane was added and a homogeneous mixture was formed. The mixture was placed at room temperature for at least 15 min to allow full interaction between the drug and the cationic surfactant. One additional volume of water and one additional volume of dichloromethane were then added. Phases were separated by centrifugation at 2,000 rpm for 5 min. The drug concentrations in the aqueous and organic

layers were estimated by UV spectrophotometry, and the partition coefficient of the IHMS ion-paired complex was calculated.

Numerous cationic surfactants were used to produce IHMS-HIP complexes, although three were chosen for further study. These three are tetraheptylammonium bromide, tetrapentylammonium bromide and tetraoctylammonuum bromide (Fluka). Other cationic lipids used to produce HIP complexes of IHMS using this method included:

trimethylalkylammonium bromide,
tetraalkylammonium bromide,
1,2-diacyl-3-trimethylammonium-propane,
dodecyltrimethylammonium bromide,
tetradecyltrimethylammonium bromide,
hexadecyltrimethylammonium bromide,
tetraethylammonium bromide,
tetrabutylammonium bromide,
1,2-dimyristoyl-3-trimethylammonium-propane,
1,2-dipalmitoyl-3-trimethylammonium-propane,
1,2-distearoyl-3-trimethylammonium-propane, and
1,2-dioleoyl-3-trimethylammonium-propane.

Following the formation of the HIP complexes using the protocol above, the complexes were purified by flash chromatography. For example, the IHMS-tetraheptylammonium complex was purified using a 200 ml flash-chromatography assembly (Sigma-Aldrich). The "flash" silica gel ("Flash," 32-63 μm, 60 Å; Scientific Adsorbant, Inc.) was packed into the column to a height of 15-22 cm. The solvent consisted of 60% 2-propanol and 40% ethanol. The samples were applied as a 20-30% solution in the solvent. Medical grade compressed nitrogen was applied to the column to maintain a flow rate of 1.5-2.5 ml/min. The eluents were collected as 5-ml aliquots and were subject to UV spectrophotometry and thin layer chromatography to identify the elution fraction containing the HIP complex, as well as estimate the drug concentration and purity. The aliquots containing pure ion-pairing complexes were saved and dried by centrifugation under vacuum.

As mentioned above, three HIP complexes were chosen for further study. The water/dichloromethane partition coefficients for IHMS-tetrapentylammonium, IHMS-tetraheptylammonium and IHMS-tetraoctylammonium HIP complexes were determined.

The results of this analysis are shown in Figure 4, as log *P*, where:

$$\log P = \log \left(\frac{[drug]_{organic\ phase}}{[drug]_{aqueous\ phase}} \right)$$

To determine the partitioning coefficients, 1 ml of a 5 mM HIP-complex solution in water and 1 ml of dichloromethane were mixed by vigorous shaking, followed by centrifugation for 5 min. Both the aqueous and organic phases were collected. The drug concentration was measured by UV spectrometry as known in the art. As indicated by the bar graph in Figure 4, the parent isoniazid and the covalently modified IHMS have negative log P values, indicating a preference for solubility in the aqueous phase. However, HIP-IHMS complexes all show preferential solubility in the organic phase, with the IHMS-tetraoctylammonium form showing the highest preference for solvation in the organic solvent. In contrast, no surfactant facilitated organic phase transfer of non-modified isoniazid.

EXAMPLE 9

Improved Efficacy of Hydrophobic Ion Paired Tetracycline

In this Example, the efficacy (*i.e.*, antimicrobial activity) of HIP-modified tetracycline was tested and compared to the activity of non-modified tetracycline, as determined in an *E. coli* growth rate assay.

A first set of experiments was designed to determine the appropriate concentration of tetracycline that would inhibit the growth of *E. coli* without completely killing the bacterial culture. The analysis of *E. coli* culture growth rates in response to free tetracycline and tetracycline-AOT complex also included control cultures that received no drug treatment and cultures that received treatment with AOT alone.

The bacterial strain used in these experiments, *E. coli* ATCC 25922, was chosen for use in this study because of its known susceptibility to tetracycline and a variety of other antimicrobials. Tetracycline hydrochloride (Sigma), AOT (Sigma), and methylene chloride (DCM; Fisher) were used as they were received from the manufacturer. The *E. coli* was cultured as a suspension in trypticase soy broth (Becton Dickinson, BBL 11768) using a

shaking water bath at 37°C and a shaker speed of 200 rpm. The suspension cultures were initiated by inoculating 1-2 colonies from an agar plate to 5 ml of liquid culture media, and allowed to grow overnight. The resulting starter culture was then used to inoculate a 250 ml shaker flask containing 95 ml of culture media. The culture was grown until it reached an optical density (OD, 600 nm) of approximately 0.75 (the cells were still in the exponential growth phase), at which time each of ten flasks were inoculated with 5 ml of the cell suspension. The growth rate was again monitored by OD readings every 20-30 minutes.

The susceptibility of *E. coli* to varying concentrations of tetracycline, the tetracycline-AOT complex, and AOT alone was determined by quantitating culture growth rates. The antimicrobial agents for testing were added to the cultures when the cell suspensions were at an OD of approximately 0.2 (*i.e.*, typically 60-75 minutes after the inoculation). After reaching this concentration, 1.0 ml of the tetracycline solution were added to the culture flasks, to produce a final concentration of tetracycline in the range of 0-24 μg/ml. Concurrently, 1.0 ml of the tetracycline-AOT complex, 1.0 ml of water (control), or 1.0 ml of AOT solution were added to separate flasks to a final concentration identical to that of the free tetracycline. Analysis of each culture condition was conducted in triplicate. Every 20-30 minutes after addition of the various drugs, a 5 ml sample was withdrawn from each flask for OD measurement and monitoring of the growth curves of the cultures.

As shown in Figure 5, free tetracycline and tetracycline-AOT complex concentrations in the range of 6-24 μg/ml completely inhibited *E. coli* growth. The one discrepancy in the data shows the highest concentration of free tetracycline (24 μg/ml) having no effect on the growth curve. It is believed that this discrepancy was due to an experimental error.

A second *E. coli* growth rate experiment, the results of which are shown in Figure 6, was run using 5.0 μg/ml concentrations of the free tetracycline and the tetracycline-AOT complex. This concentration was also still high enough to completely stop all growth of *E. coli*.

A third *E. coli* growth rate experiment, the results of which are shown in Figure 7, was run using 0.6 μg/ml concentrations of the free tetracycline and the tetracycline-AOT complex. As shown in this Figure, the ion-paired tetracycline inhibited the growth rate of the *E. coli* culture 45% more effectively than the free tetracycline. To demonstrate that the

growth inhibition was not caused by the AOT moiety, a control flask was prepared in which AOT solution was added to a final concentration of 1.0 μg/ml. Another control culture received the same volume of water. The growth rate of the AOT-treated and free tetracycline-treated cultures were not significantly different from the water-treated control culture. Drug was added to these cultures after 60 minutes of incubation. Almost immediately following the addition of the tetracycline-AOT complex, a reduction in growth rate was observed for this culture.

Another experiment, the results of which are shown in Figure 8, was performed using a slightly higher concentration of drug to determine a specific dose of free tetracycline that would inhibit *E. coli* growth similar to the growth suppression observed using the ion-paired tetracycline at a concentration of 0.6 μg/ml. However, as indicated in this Figure, the elevated concentration of 1.0 μg/ml of both the free tetracycline and the ion-paired tetracycline caused similar inhibition of the growth rate. This suggested that an intermediate concentration between 0.6-1.0 μg/ml is optimal. It can also be seen that AOT alone does not inhibit the growth rate of the culture, thus demonstrating that the increased efficacy of the tetracycline-AOT complex is due to the antibiotic-AOT complex and not due to the presence of the AOT-moiety.

In other experiments, an HIP-complexed form of cisplatin was shown to be more effective at inhibiting target cells in an *in vitro* culture system than a non-complexed form of the drug.

These experiments demonstrate that using the HIP complexed pharmaceuticals are effective at improving the efficacy of the pharmaceutical. As indicated above, it is intended that the present invention will find use with numerous pharmaceuticals (including antimicrobials) and alternative HIP reagents. Thus, it is not intended that the present invention be limited to any particular pharmaceutical and/or HIP complex.

EXAMPLE 10

Improved Efficacy of Hydrophobic Ion Paired Cisplatin

This Example describes the improved efficacy of the cisplatin-AOT complex compared to non-complexed cisplatin, as determined using a tissue culture model system. Specifically, the intracellular concentrations of platinum were measured following treatment of cultured mammalian cells with cisplatin or cisplatin-AOT, and the cytotoxic activities of the cisplatin forms were compared using a cell culture cytotoxicity assay. The mammalian

cells used in this Example were cultured using standard culture techniques and conditions well known in the art (*e.g.*, cells were grown in α-MEM culture medium containing 10% fetal bovine serum at 37°C in 5% CO_2). Mammalian cell culture reagents are widely available from numerous manufacturers, including, but not limited to, GibcoBRL and Sigma. The AOT-cisplatin complex was synthesized as described in Example 3.

To measure the intracellular accumulation (*i.e.*, bioavailability) of platinum following treatment with cisplatin or cisplatin-AOT, Chinese hamster ovary (CHO) cells were plated into culture dishes at a density of $1x10^6$ cells/ml, where each dish received 3 ml of the cell suspension. Following establishment of the adherent culture, the cells were either exposed to cisplatin at a concentration of 200 μM or exposed to the cisplatin-AOT complex at a concentration of 50 μM for one hour. To quantitate the intracellular levels of platinum, the CHO cells were harvested and normalized to a density of $1x10^7$ cells/ml before analysis by atomic absorption (AA) spectrometry. The cell culture exposed to 200 μM cisplatin showed an intracellular platinum concentration of $1x10^{-5}$ μM, while the culture exposed to 50 μM AOT-cisplatin showed an intracellular platinum concentration of $5x10^{-5}$ μM.

In a similar experiment, the results of which are shown graphically in Figure 9, separate cultures of CHO cells were exposed to 100 μM concentrations of either cisplatin or cisplatin-AOT, for one hour. After this period, the cells exposed to cisplatin showed an intracellular concentration of platinum of 1.32 ± 0.27 picomoles/10^7 cells, while cells exposed to the cisplatin-AOT complex showed an intracellular concentration of platinum of 5.26 ± 1.26 picomoles platinum/10^7 cells.

Thus, the cisplatin-AOT complex demonstrates at least a four-fold enhancement in the ability to accumulate in the intracellular environment of cultured mammalian cells compared to the non-complexed form of cisplatin.

To assess the cytotoxic activities of cisplatin and AOT-cisplatin, a plating efficiency/cell survival assay was used. In this assay, CHO cell cultures were exposed to cisplatin or AOT-cisplatin at concentrations of 1, 5, 10, 20, 50, 75 or 100 μM for one hour. Prior to adding the drugs to the cultures, cisplatin and cisplatin-AOT were dissolved in DMSO, and the concentrations were determined based on the correction of platinum concentration, as detected by atomic absorption spectrometry. Following this incubation in the presence of drug, the cells were harvested, counted, and replated at a final density of 100-500 cells per dish for each drug and exposure concentration. These cultures were

allowed to grow an additional 7 days, after which time they were stained with crystal violet (2% crystal violet in ethanol).

Colonies in each dish were counted and the plating efficiency for each different exposure was calculated by comparing the colony number after 7 days to the number of cells used to initiate the culture. Cell survival rate in each exposure group was then calculated based on the plating efficiency of cisplatin-AOT exposed cells compared to that obtained from the control group (*i.e.*, the cisplatin exposed cells).

The plating efficiency data for the cisplatin and cisplatin-AOT exposed cultures is shown graphically in Figure 10. Based on these plating efficiency assays, the LD_{50} (50% of lethal dose) for cisplatin in this experimental system was 22 μM, while the LD_{50} for cisplatin-AOT was 4.5 μM. Furthermore, the plating efficiency of cultures treated with cisplatin-AOT was consistently and significantly lower than cultures treated with cisplatin at practically the full range of drug concentrations. From these data, it is concluded that the cisplatin-AOT drug has greater cytotoxic activity than the parent cisplatin.

EXAMPLE 11

Efficacy Study of Hydrophobic Ion Paired Cisplatin Delivered in a Poloxamer Gel *in vivo*

This Example provides a protocol for the incorporation of HIP-complexed cisplatin described in Example 3 into a poloxamer gel delivery vehicle. In addition, this Example provides a protocol for analyzing the efficacy of the HIP-cisplatin complex *in vivo* compared to non-HIP-complexed cisplatin, using a mouse tumor model.

Poloxamer 407 (BASF) is dissolved in DI water (22%, w/w) at 4°C and filter sterilized through a 0.20 μm cellulose acetate membrane (Life Science Products). Cisplatin (Aldrich) is then suspended in the poloxamer solution at a final concentration of 100 μM. Cisplatin-AOT (described in Example 3) is suspended into dimethyl sulfoxide (DMSO) and then suspended into the poloxamer solution at a final cisplatin concentration of 100 μM.

Female C3H-HeJ mice aged 6-8 weeks are injected with a mouse mammary carcinoma cell line, Gollin-B at a concentration of 0.5 million cells per mouse into the flank fat pad. When a tumor arising from the injected cells reaches 7 mm in any direction, the tumor is surgically excised.

The surgically-treated mice are then divided into four groups, and treated as follows. Group I receives no treatment; Group II is injected with 0.2 ml poloxamer solution only

(with no incorporated drug) at the surgical site; Group III is injected with 0.2 ml poloxamer solution containing cisplatin at the surgical site; and Group IV is injected with 0.2 ml poloxamer solution containing the cisplatin-AOT complex at the surgical site.

Regrowth of the tumors is monitored in the Group I control mice. When the regrown tumor reaches 7 mm in any direction in these control mice, the animals in all the groups are sacrificed, and tissue from the liver, kidney, small intestine, brain, and the original surgical site is collected for histopathology examination. The tumor reoccurrence rate and metastasis are assessed for each treatment group.

EXAMPLE 12

Subcutaneous Delivery of Hydrophobic Ion Paired Vancomycin in a Poloxamer Gel and Assessment of Plasma Drug Levels

This Example describes the formation of a poloxamer gel containing the vancomycin HIP-complex described in Example 2, and the drug release characteristics from the poloxamer gel in an *in vivo* mouse model system.

Three clean and dry glass tubes (oven dried at 60°C for 1 hr and allowed to cool to room temperature) each received 65 mg of dried HIP-vancomycin powder, corresponding to 50 mg of the vancomycin pharmacophore, according to the following calculation:

MW Vancomycin = 1486 - 35.45 (chloride ion) = 1450 g/mol
MW AOT = 444.55 - 22.99 (sodium ion) = 421.56 g/mol

Thus, MW vancomycin-HIP complex = 1872.11 g/mol, so the weight percentage of vancomycin pharmacophore within the HIP complex is: (1450.55)/(1872.11), or 77.5%

To each glass tube was added a solution of poloxamer 407 (BASF; 22% w/w in water) to bring the final concentration of the HIP-vancomycin complex to 10 mg/ml (adjusted weight to normalize the concentration of vancomycin in the HIP complex and the hydrochloride form).

In order to study the drug release properties of the vancomycin and HIP-vancomycin from a poloxamer gel, an *in vivo* mouse model system was used, in which the systemic concentration of vancomycin was monitored following subcutaneous administration of the polox-vancomycin (*i.e.*, HIP-vancomycin poloxamer gel) formulations.

Thirty-six C3H-HeJ mice were divided into three groups of 12 mice each. These groups were treated as follows:

Group I: given a subcutaneous injection of non-complexed vancomycin in a poloxamer solution into the mouse flank fat pad;

Group II: given a subcutaneous injection of HIP-complexed vancomycin suspended in DI water;

Group III: given a subcutaneous injection of HIP-complexed vancomycin in poloxamer solution.

The dose level of vancomycin delivered to each mouse was 100 mg vancomycin per kg of mouse weight. Calculation of the vancomycin to be administered to each mouse was corrected for the difference in molecular weights between the complexed and non-complexed vancomycin, such that each mouse received the same concentration of "parent" vancomycin per kg of mouse weight using the calculation described above.

Three animals from each group were sacrificed at days 1, 2, 4 and 8. The vancomycin levels in the plasma were assayed using a TDx clinical analyzer (Abbott Laboratories). The TDx instrument detects fluorescence polarization of a fluorescently-tagged, polyclonal antibody specific for the analyte in question (*i.e.*, an anti-vancomycin antibody). The larger the signal, the greater the concentration of vancomycin.

The results of this assay are shown in Figure 11. As indicated in this Figure, poloxamer delivery of HIP-complexed vancomycin provides a sustained plasma concentration of vancomycin over at least eight days following injection of the poloxamer matrix.

EXAMPLE 13

Microscopic Tissue Analysis and *in vivo* Tissue Concentrations of Hydrophobic Ion Paired Vancomycin Delivered from a Poloxamer Gel

This Example provides protocols for the analysis of vancomycin distribution in mouse tissues following delivery of vancomycin or HIP-vancomycin from a poloxamer gel.

The tissue distribution of vancomycin following poloxamer delivery of vancomycin or HIP-vancomycin is assessed using an immuno-gold silver enhancing immunostaining method to visualize the vancomycin within tissue sections. In preferred embodiments, HIP-vancomycin is synthesized as described in Example 2. Vancomycin and HIP-vancomycin are incorporated into poloxamer solutions, which are delivered subcutaneously or

intraperitoneally to mice. Following a suitable time period, the mice are sacrificed, tissues are harvested, and slides suitable for analysis by immunochemistry are prepared. Tissue samples from muscle, liver, lung and kidney, as well as other tissues, are collected for analysis.

The slides are incubated with a commercially available anti-vancomycin primary antibody for one hour at 37°C, followed by two washes with PBS. Slides are then incubated with a suitable secondary antibody (preferably gold conjugated) specific to the primary antibody. The slides are then visualized using a silver-enhancing kit (Sigma). Slides are observed under light microscopy. Light microscopy images are also be quantitated for vancomycin concentration.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art of cell biology, medicine, medicinal chemistry, and/or related fields are intended to be within the scope of the present invention.

CLAIMS

We claim:

1. A method for preparing a composition for the controlled-release of a pharmaceutical, comprising the steps of:

a) providing:

i) a pharmaceutical,

ii) an amphiphilic counterion;

b) complexing said pharmaceutical with said amphiphilic counterion in an aqueous solution under conditions wherein said pharmaceutical and said counterion form a hydrophobic ion-paired complex with reduced aqueous-solubility; and

c) isolating said hydrophobic ion-paired complex to yield a composition for the controlled release of said pharmaceutical.

2. The method of Claim 1, further comprising the step of:

d) drying said hydrophobic ion-paired complex to form a hydrophobic ion-paired pharmaceutical powder.

3. The composition produced by the method of Claim 1.

4. The method of Claim 1, wherein said pharmaceutical is an antimicrobial.

5. The method of Claim 1, wherein said pharmaceutical is selected from the group consisting of vancomycin, tetracycline, tacrine, L-phenylephrine, cisplatin, isoniazid and isoniazid methanesulfonate.

6. The method of Claim 1, wherein said amphiphilic counterion is selected from the group consisting of bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate, sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide, palmitoylcholine chloride, palmitoyl carnitine chloride, ZWITTERGENT 3-16,

polyethylene oxide polymer, tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide, trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane, 1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, and 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

7. A composition for pharmaceutical delivery comprising the composition of Claim 3 and a poloxamer solution having reverse thermal gelation properties.

8. The composition of Claim 7, wherein said poloxamer is poloxamer 407.

9. A method for preparing a hydrophobic ion-paired complex comprising a pharmaceutical, comprising the steps of:

a) providing:

i) a pharmaceutical, wherein said pharmaceutical is selected from the group consisting of neutral pharmaceuticals and zwitterionic pharmaceuticals,

ii) an amphiphilic counterion,

iii) a metal ion;

b) complexing said pharmaceutical with said metal ion to produce a pharmaceutical-metal ion complex;

c) combining said pharmaceutical-metal ion complex with said amphiphilic counterion in an aqueous solution under conditions wherein said pharmaceutical and said counterion form a hydrophobic ion-paired complex with reduced aqueous-solubility; and

d) isolating said hydrophobic ion-paired complex.

10. The method of Claim 10, further comprising the step of:

e) drying said hydrophobic ion-paired complex to form a hydrophobic ion-paired pharmaceutical powder.

11. The method of Claim 10, wherein said amphiphilic counterion is selected from the group consisting of bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate, sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide, palmitoylcholine chloride, palmitoyl carnitine chloride, ZWITTERGENT 3-16, polyethylene oxide polymer, tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide, trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane, 1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, and 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

12. The method of Claim 10, wherein said pharmaceutical is isoniazid.

13. The method of Claim 10, wherein said pharmaceutical is a polypeptide.

14. The method of Claim 10, wherein said metal ion is multivalent.

15. The method of Claim 10, wherein said metal ion is divalent.

16. The method of Claim 15, wherein said metal ion is selected from the group consisting of calcium and zinc.

17. The hydrophobic ion paired complex produced by the method of Claim 10.

18. A composition for pharmaceutical delivery comprising the hydrophobic ion paired complex of Claim 18 and a poloxamer solution having reverse thermal gelation properties.

19. The composition of Claim 18, wherein said poloxamer is poloxamer 407.

20. An isolated hydrophobic ion-paired complex comprising an antimicrobial.

21. The hydrophobic ion-paired complex of Claim 20, wherein said complex is dried to form a hydrophobic pharmaceutical powder.

22. The hydrophobic ion-paired complex of Claim 20, wherein said antimicrobial is selected from the group consisting of tetracycline, vancomycin, isoniazid and isoniazid methanesulfonate.

23. A composition comprising the hydrophobic ion-paired complex of Claim 20 and a poloxamer solution having reverse thermal gelation properties.

24. The composition of Claim 23, wherein said poloxamer is poloxamer 407.

25. A method for producing a pharmaceutical with improved efficacy, comprising the steps of:

a) providing:
   i) a pharmaceutical,
   ii) an amphiphilic counterion;
b) complexing said pharmaceutical with said amphiphilic counterion in an aqueous solution under conditions wherein said pharmaceutical and said counterion form a hydrophobic ion-paired complex with reduced aqueous-solubility; and
c) isolating said hydrophobic ion-paired complex to yield a pharmaceutical with improved efficacy.

26. The method of Claim 28, further comprising:

d) drying said hydrophobic ion-paired complex to form a hydrophobic ion-paired pharmaceutical powder.

27. The pharmaceutical with improved efficacy produced by the method of Claim 28.

28. The pharmaceutical of Claim 30, wherein said pharmaceutical is selected from the group consisting of anti-cancer agents and antimicrobials.

29. The method of Claim 28, wherein said pharmaceutical is selected from the group consisting of vancomycin, tetracycline, tacrine, L-phenylephrine, cisplatin, isoniazid and isoniazid methanesulfonate.

30. The method of Claim 28, wherein said amphiphilic counterion is selected from the group consisting of bis(2-ethylhexyl) sodium sulfosuccinate, sodium dodecyl sulfate, sodium octyl sulfate, sodium tetradecyl sulfate, sodium octadecyl sulfate, cholesterol sulfate, sodium laurate, cholesterol sulfate, sodium dodecyl sulfonate, sodium decyl sulfonate, sodium octyl sulfonate, sodium oleate, 1-heptanesulfonic acid, cetyl trimethyl ammonium bromide, palmitoylcholine chloride, palmitoyl carnitine chloride, ZWITTERGENT 3-16, polyethylene oxide polymer, tetraheptylammonium bromide, tetrapentylammonium bromide, tetraoctylammonuum bromide, trimethylalkylammonium bromide, tetraalkylammonium bromide,1,2-diacyl-3-trimethylammonium-propane, dodecyltrimethylammonium bromide,tetradecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, tetraethylammonium bromide, tetrabutylammonium bromide, 1,2-dimyristoyl-3-trimethylammonium-propane, 1,2-dipalmitoyl-3-trimethylammonium-propane, 1,2-distearoyl-3-trimethylammonium-propane, and 1,2-dioleoyl-3-trimethylammonium-propane, long chain fatty acids, arginine octyl ester, arginine dodecyl ester, and ionic forms and dissociation products of the foregoing.

31. A composition for pharmaceutical delivery comprising the pharmaceutical of Claim 30 and a poloxamer solution having reverse thermal gelation properties.

32. The composition of Claim 31, wherein said poloxamer is poloxamer 407.

33. A method for treating a subject,

a) providing:

i) a hydrophobic ion-paired complex,

ii) a poloxamer solution, and

iv) a subject;

b) combining said poloxamer solution with said hydrophobic ion-paired complex to form a mixture, under conditions where said mixture is liquid; and

c) applying said liquid mixture to said subject, under conditions wherein said poloxamer-HIP mixture becomes a gel.

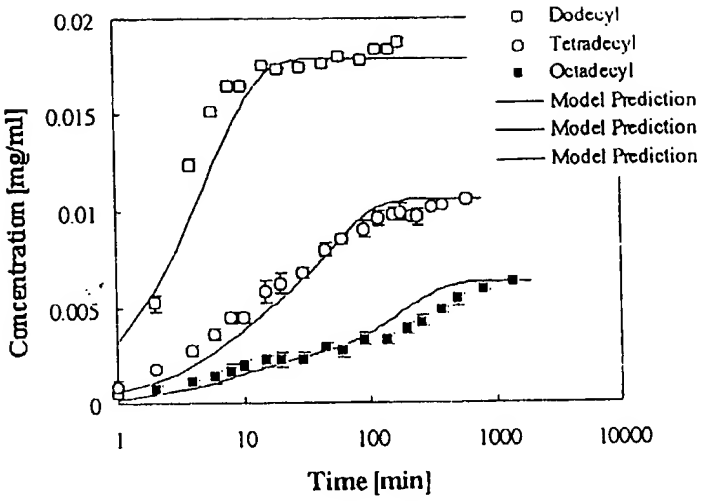
34. The method of Claim 33, wherein said hydrophobic ion-paired complex comprises an antimicrobial.

35. The method of Claim 34, wherein said hydrophobic ion-paired complex comprises an antimicrobial selected from the group consisting of tetracycline, vancomycin, isoniazid and isoniazid methanesulfonate.

36. The method of Claim 33, wherein said liquid mixture is applied to a localized region of said subject.

37. The method of Claim 36, wherein said localized region of said subject is selected from the group consisting of diabetic ulcers, surgical incision sites, and wounds.

FIGURE 1



Dissolution profiles for ion-paired tacrine complexes in PBS [0.1-M] with model predicted release profiles

FIGURE 2

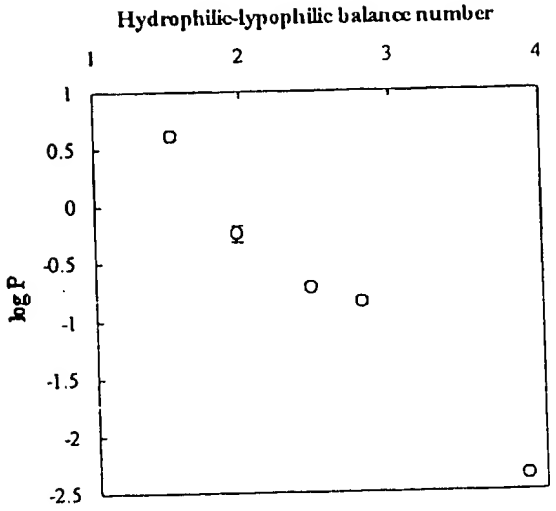
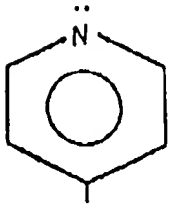
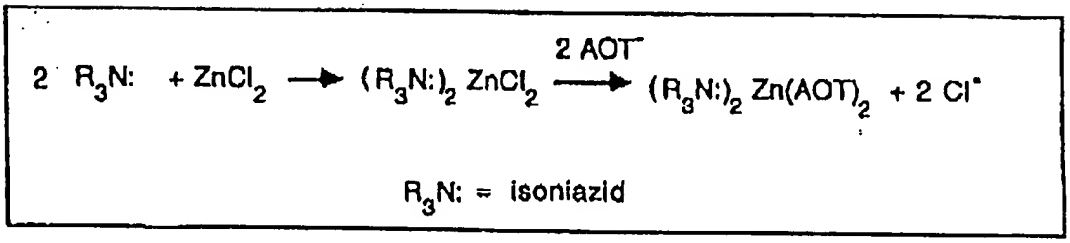
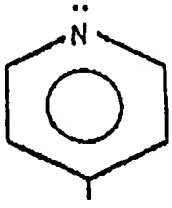

Hydrophilic-lypophilic balance number
1
2
3
4
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
log P

FIGURE 3



Structure of isoniazid (isonicotinic acid hydrazide)





Structure of isoniazid methanesulfonate (IHMS)

FIGURE 4

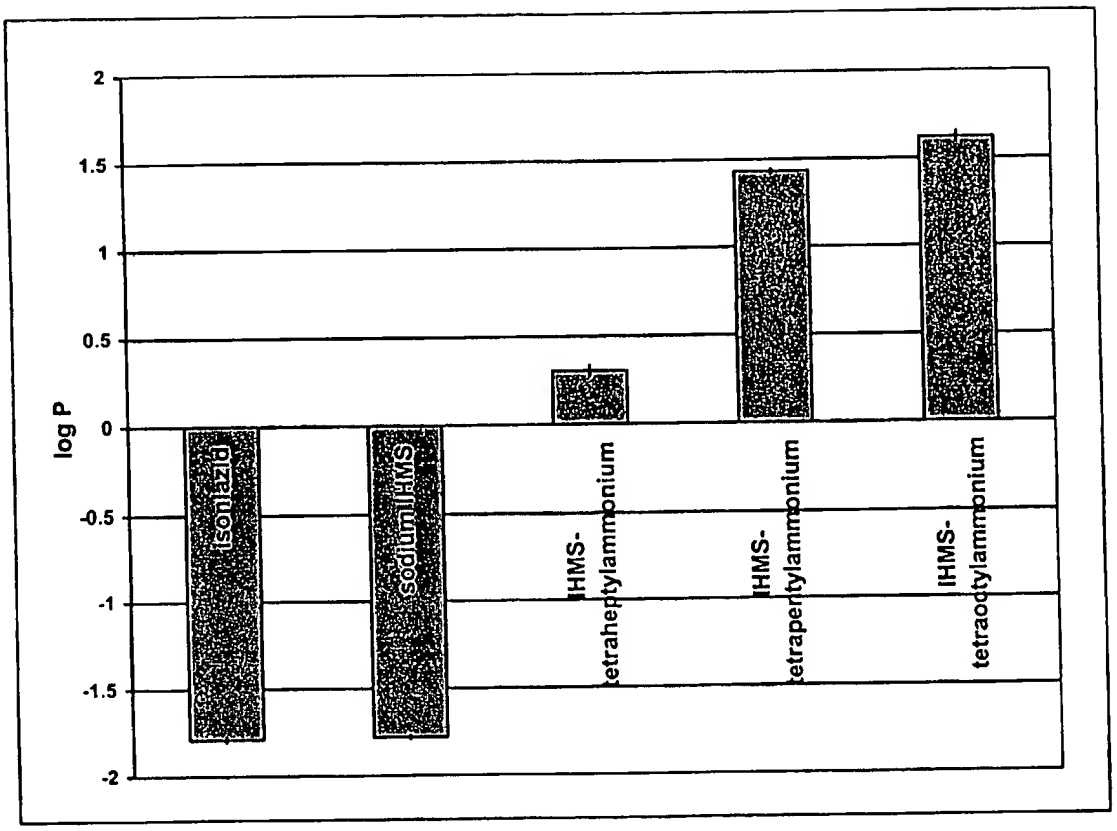

log P
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
isoniazid
sodium IHMS
IHMS-tetraheptylammonium
IHMS-tetrapentylammonium
IHMS-tetraoctylammonium

FIGURE 5

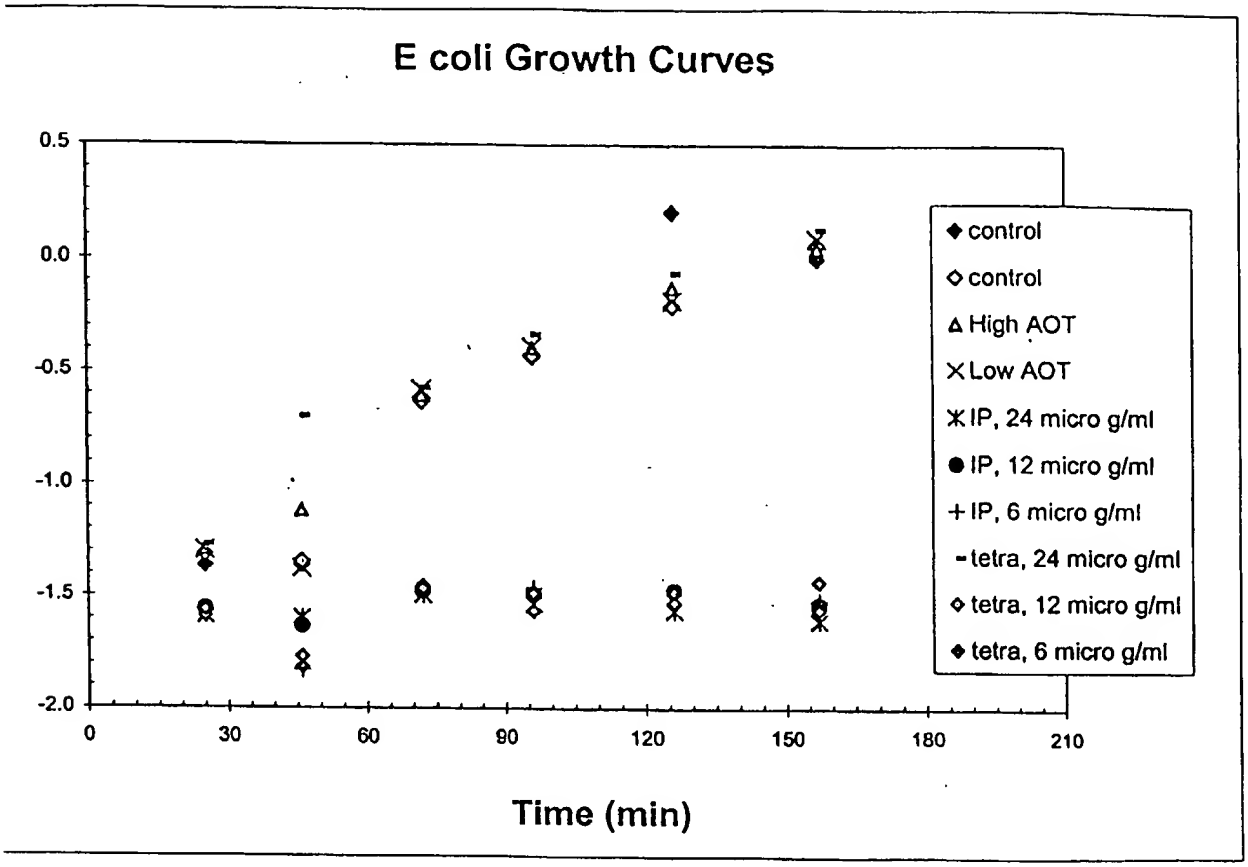

E coli Growth Curves
0.5
0.0
-0.5
-1.0
-1.5
-2.0
0
30
60
90
120
150
180
210
Time (min)
control
control
High AOT
Low AOT
IP, 24 micro g/ml
IP, 12 micro g/ml
IP, 6 micro g/ml
tetra, 24 micro g/ml
tetra, 12 micro g/ml
tetra, 6 micro g/ml

FIGURE 6

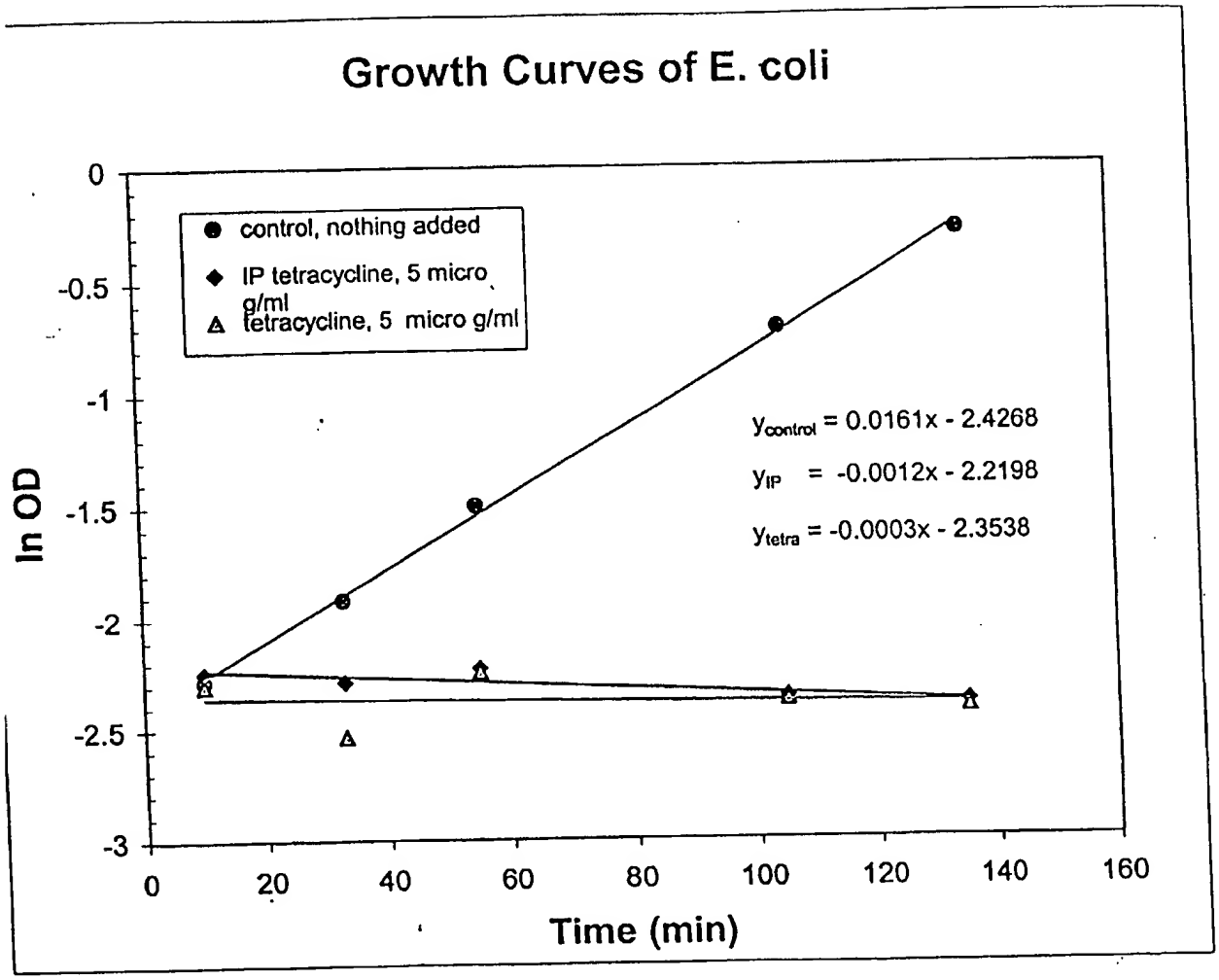

Growth Curves of E. coli
control, nothing added
IP tetracycline, 5 micro g/ml
tetracycline, 5 micro g/ml
$y_{control}$ = 0.0161x - 2.4268
y_{IP} = -0.0012x - 2.2198
y_{tetra} = -0.0003x - 2.3538
ln OD
0
-0.5
-1
-1.5
-2
-2.5
-3
0
20
40
60
80
100
120
140
160
Time (min)

FIGURE 7

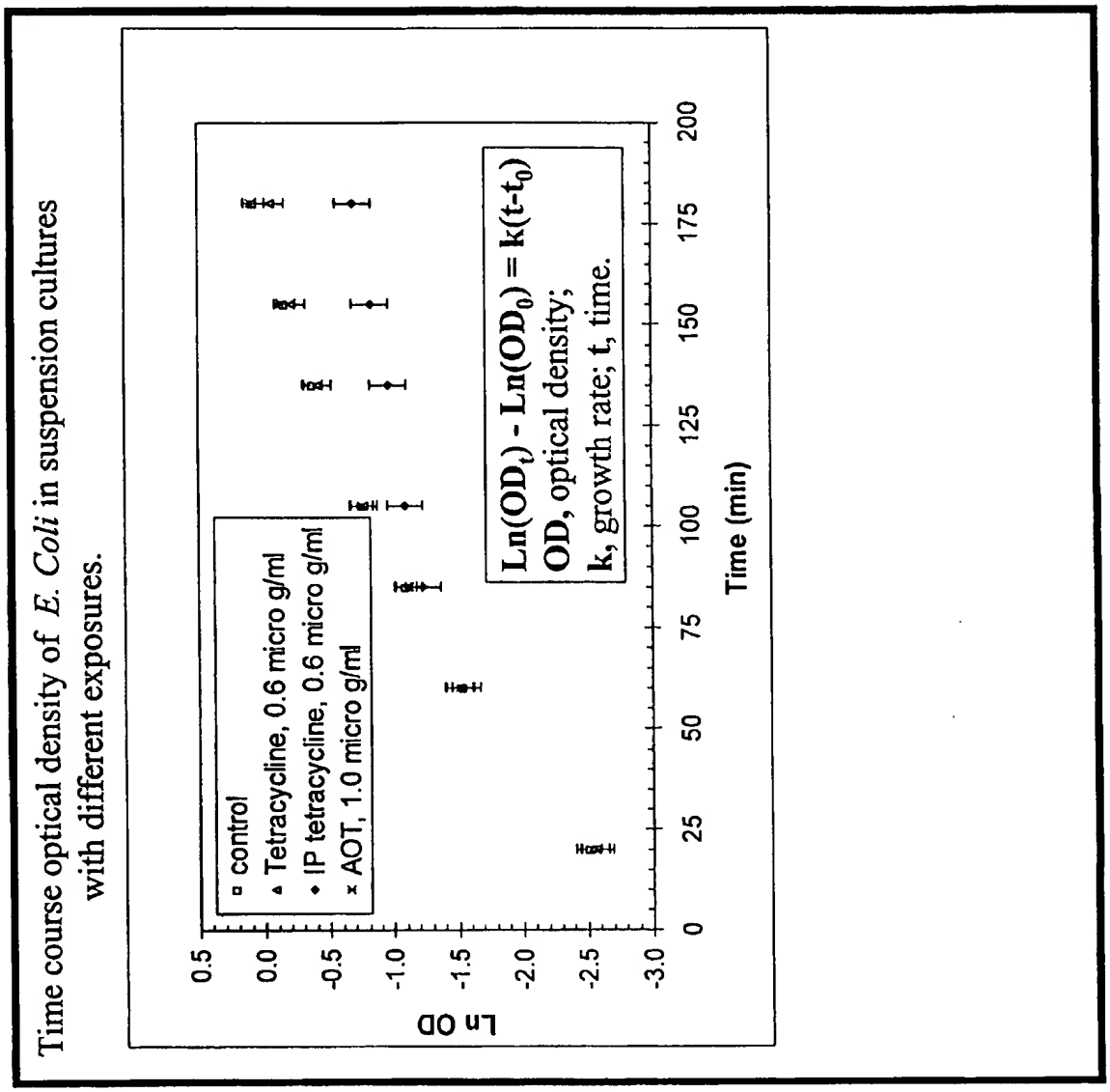

Time course optical density of E. Coli in suspension cultures with different exposures.
Ln OD
Time (min)
control
Tetracycline, 0.6 micro g/ml
IP tetracycline, 0.6 micro g/ml
AOT, 1.0 micro g/ml
Ln(OD_t) - Ln(OD_0) = k(t-t_0)
OD, optical density;
k, growth rate; t, time.
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
25
50
75
100
125
150
175
200

FIGURE 8

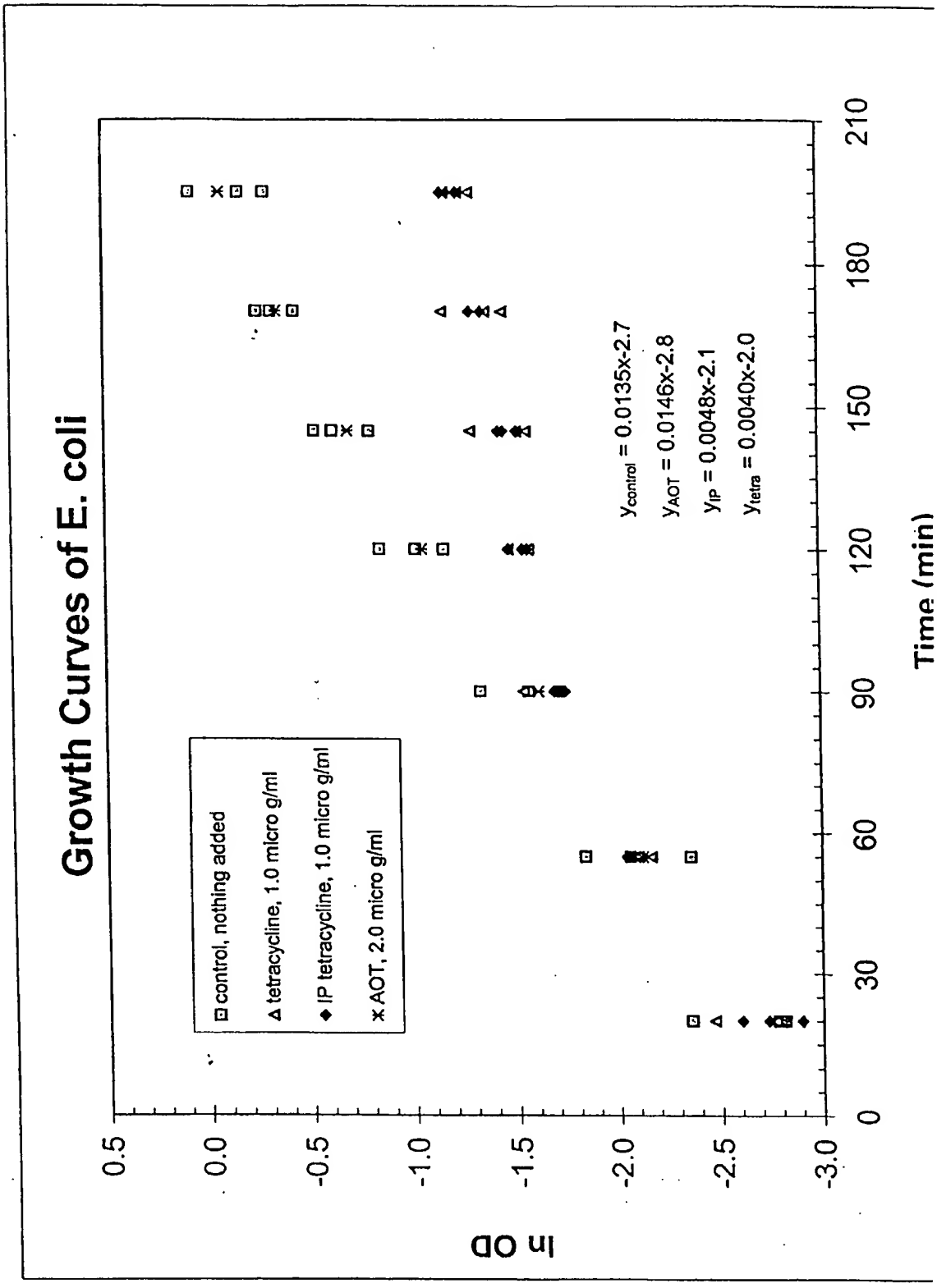

Growth Curves of E. coli
control, nothing added
tetracycline, 1.0 micro g/ml
IP tetracycline, 1.0 micro g/ml
AOT, 2.0 micro g/ml
$y_{control}$ = 0.0135x-2.7
y_{AOT} = 0.0146x-2.8
y_{IP} = 0.0048x-2.1
y_{tetra} = 0.0040x-2.0
ln OD
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
30
60
90
120
150
180
210
Time (min)

FIGURE 9

Cellular concentration of Pt in CHO cells exposed to cisplatin and the AOT complex of cisplatin.

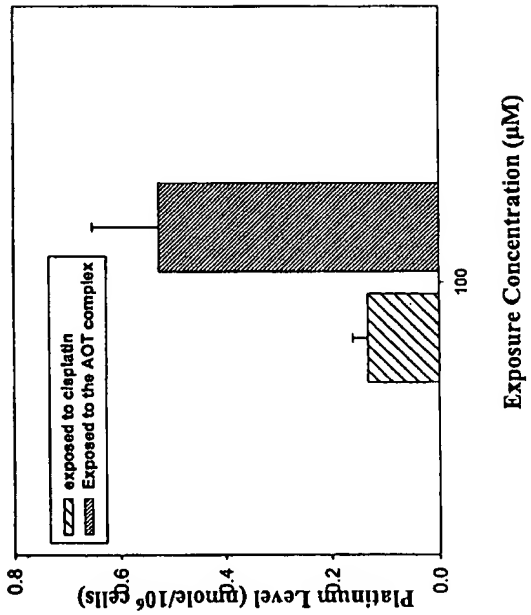


FIGURE 10

CHO cell survival rates after exposure to cisplatin or the AOT complex of cisplatin.

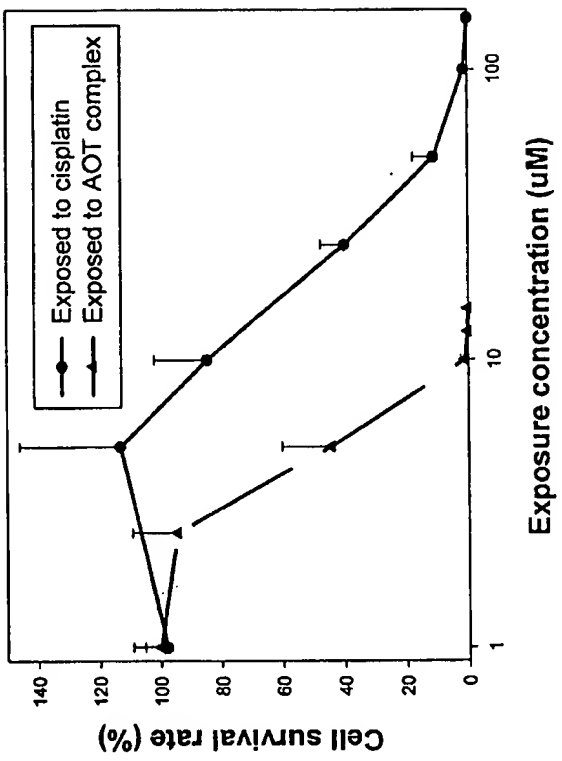


FIGURE 11

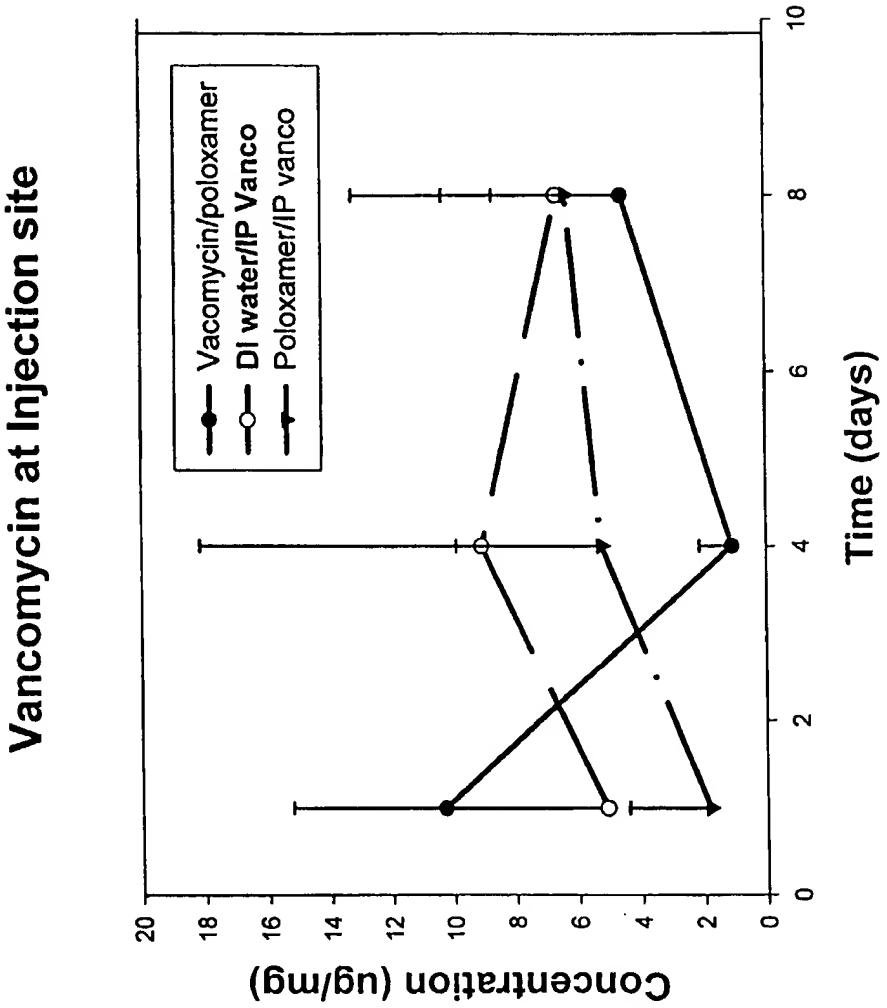

Vancomycin at Injection site
Vacomycin/poloxamer
DI water/IP Vanco
Poloxamer/IP vanco
Concentration (ug/mg)
Time (days)
0
2
4
6
8
10
12
14
16
18
20

FIGURE 12

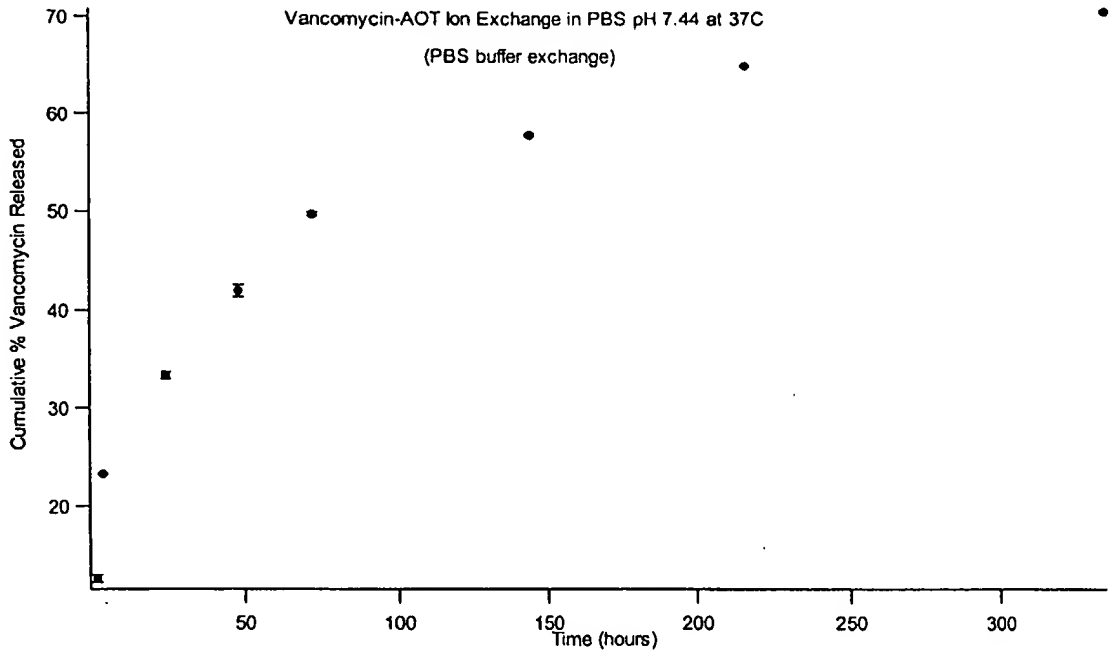
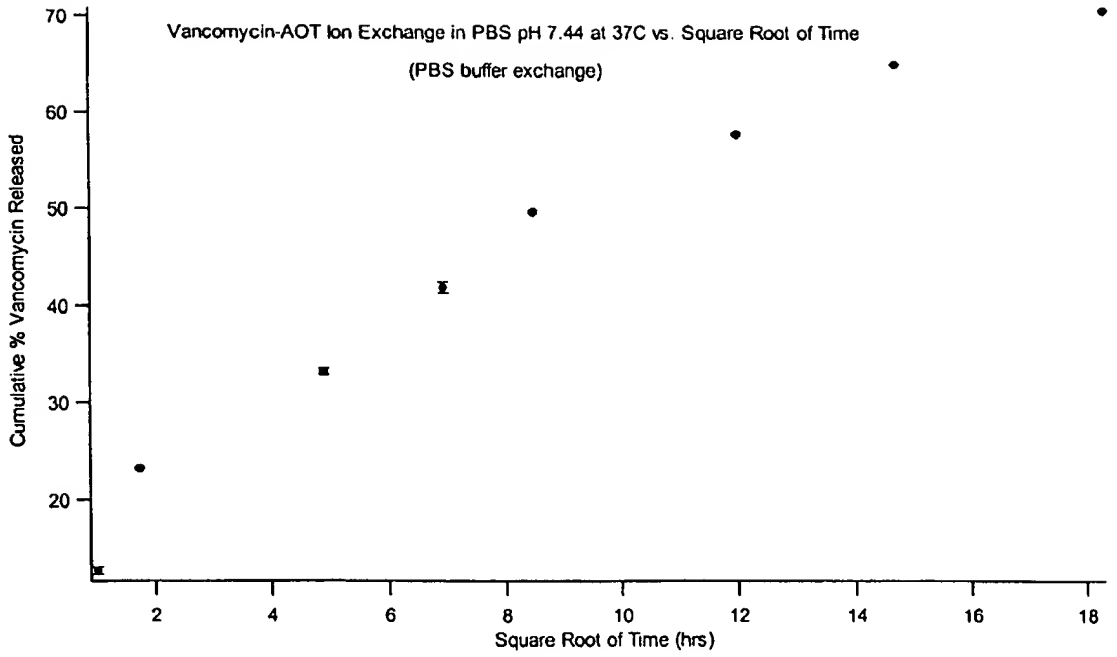

Vancomycin-AOT Ion Exchange in PBS pH 7.44 at 37C
(PBS buffer exchange)
Cumulative % Vancomycin Released
70
60
50
40
30
20
50
100
150
200
250
300
Time (hours)

FIGURE 13


Vancomycin-AOT Ion Exchange in PBS pH 7.44 at 37C vs. Square Root of Time
(PBS buffer exchange)
Cumulative % Vancomycin Released
70
60
50
40
30
20
2
4
6
8
10
12
14
16
18
Square Root of Time (hrs)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30424

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 47/30
US CL :514/772.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/772.2, 2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,770,559 A (MANNING et al) 23 June 1998, abstract, column 3, line 4 to column 9 and line 53, column 10, line 63 to column 11 and line 65, column 14, lines 9-57, examples 1, 9, 19-32 and claims 1-4, 10, 11 and 22-26.	1-6, 9-22, 25-30 and 33-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 JANUARY 2001	Date of mailing of the international search report 09 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BLESSING FUBARA Telephone No. (703) 308-0196

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